IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re the Application of:	)	Group Art Unit: 1644
LAHN et al.	)	Examiner: Schwadron, R.B.
Serial No.: 09/826,319	)	Confirmation No.: 4155
Filed: April 3, 2001	)	
Atty. File No.: 2879-80	)	**APPEAL BRIEF**
For: "METHOD TO INHIBIT AIRWAY HYPERRESPONSIVENESS USING AEROSOLIZED T CELL RECEPTOR ANTIBODIES"	)	***SUBMITTED VIA EFS-WEB***

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313

Dear Sir:

This Appeal Brief is filed in response to the non-final Office Action mailed on April 6, 2007. A Notice of Appeal was filed in the above-identified application on July 6, 2007. The previously paid fee in the amount of $250 will be applied to this Appeal Brief as stated in the Office Action mailed April 6, 2007. Accordingly, the filing of this Appeal Brief is believed to be timely and no additional fee is believed to be required. Please credit any overpayment or debit any underpayment to Deposit Account 19-1970.

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I. REAL PARTY IN INTEREST

The real party in interest is **National Jewish Medical and Research Center**, the assignee of record, the Assignment being recorded with the United States Patent Office at Reel/Frame 012070/0760.

II. RELATED APPEALS AND INTERFERENCES

A prior Appeal Brief was filed in this application on November 20, 2006. The Appeal was dismissed by reopening of prosecution.

III. STATUS OF CLAIMS

The status of the claims in the application is:

A. TOTAL NUMBER OF CLAIMS IN THE APPLICATION

Claims in the application are: 1-36

B. CURRENT STATUS OF THE CLAIMS:

1. Claims cancelled: 33
2. Claims withdrawn: 3-8
3. Claims pending: 1-32 and 34-36
4. Claims allowed: None
5. Claims rejected: 1, 2, 9-32 and 34-36

C. CLAIMS ON APPEAL

The claims on appeal are: 1, 2, 9-32 and 34-36.

IV. STATUS OF AMENDMENTS

No amendment was filed subsequent to the final rejection mailed on February 22, 2006 or subsequent to the non-final Office Action mailed April 6, 2007.

V. SUMMARY OF CLAIMED SUBJECT MATTER

Claim 1

Claim 1 is directed to method to reduce airway hyperresponsiveness line in a mammal that has, or is at risk of developing, airway hyperresponsiveness. (Specification: page 9, lines 2-5; page 18, line 27 to page 19, line 26; page 20, lines 5-17)

The method includes the step of administering to the lungs of the mammal an aerosolized antibody formulation comprising antibodies that selectively bind to a receptor on a T cell selected from: a T cell antigen receptor (TCR) selected from the an αβ TCR and a γδ TCR, CD3, CD4 and CD8. (Specification: page 11, lines 1-22; page 13, lines 12-17; page 37, lines 20-27)

The binding of the antibodies to the receptor causes the depletion or inactivation of the T cell. (Specification: page 9, line 23 to page 10, line 9; page 11, lines 24-27; Figs. 2A-2B; Figs. 3A-3B).

The administration of the antibody formulation reduces airway hyperresponsiveness in said mammal. (Specification: page 17, line 18 to page 18, line 26; Example 1, Example 2, Example 3; Figs. 1A-1F)

The administration of the aerosolized antibody formulation affects pulmonary T cell responses in the mammal, while peripheral T cell responses in the mammal are neither substantially stimulated nor substantially inhibited. (Specification: page 10, lines 10-18; Example 5; Fig. 3A-3B).

Claim 2

Claim 2 is directed to a method to reduce airway hyperresponsiveness line in a mammal that has, or is at risk of developing, airway hyperresponsiveness. (Specification: page 9, lines 2-5; page 18, line 27 to page 19, line 26; page 20, lines 5-17)

The method includes the step of administering to the lungs of the mammal an aerosolized antibody formulation comprising antibodies that selectively bind to an αβ T cell receptor (TCR). (Specification: page 11, lines 1-22; page 12, lines 4-13 page 13, lines 12-17; page 37, lines 20-27)

The binding of the antibodies to the receptor causes the depletion or inactivation of the T cell. (Specification: page 9, line 23 to page 10, line 9; page 11, lines 24-27; Figs. 2A-2B).

The administration of the antibody formulation reduces airway hyperresponsiveness in said mammal. (Specification: page 17, line 18 to page 18, line 26; Example 1; Figs. 1A-1F)

The administration of the aerosolized antibody formulation affects pulmonary T cell responses in the mammal, while peripheral T cell responses in the mammal are neither substantially stimulated nor substantially inhibited. (Specification: page 10, lines 10-18).

<u>Claim 16 and Claims 19-23</u>

Claims 16 and 19-23 are directed to particular embodiments of the invention, wherein said aerosolized antibody formulation is administered at low doses (Specification: Page 10, lines 22-28; page 34, line 9 to page 35, line 6), including a dose of between about 5 μg antibody and about 10 μg antibody per milliliter of formulation (Claim 16), or less than about 40 μg x kilogram^{-1} body weight of the mammal (Claim 19), less than about 1 μg x kilogram^{-1} body weight of the mammal (Claim 20), less than about 0.5 μg x kilogram^{-1} body weight of the mammal (Claim 21), less than about 0.1 μg x kilogram^{-1} body weight of said mammal (Claim 22), or less than about 20 ng x kilogram^{-1} body weight of the mammal (Claim 23). (Specification: page 6, line 26 to page 7, line 2; page 35, lines 7-28; page 36, lines 20-21; original Claims 16, and 19-23)

<u>Claim 36</u>

Claim 36 is directed to method to reduce airway hyperresponsiveness line in a mammal that has, or is at risk of developing, airway hyperresponsiveness. (Specification: page 9, lines 2-5; page 18, line 27 to page 19, line 26; page 20, lines 5-17)

The method includes the step of administering to the lungs of the mammal an aerosolized antibody formulation comprising antibodies that selectively bind to a receptor on a T cell selected from: a T cell antigen receptor (TCR) selected from the an αβ TCR and a γδ TCR, CD3, CD4 and CD8. (Specification: page 11, lines 1-22; page 13, lines 12-17; page 37, lines 20-27)

The binding of the antibodies to the receptor causes the depletion or inactivation of the T cell. (Specification: page 9, line 23 to page 10, line 9; page 11, lines 24-27; Figs. 2A-2B; Figs. 3A-3B).

The administration of the antibody formulation reduces airway hyperresponsiveness in said mammal. (Specification: page 17, line 18 to page 18, line 26; Example 1, Example 2, Example 3; Figs. 1A-1F)

In this method, any stimulation or inhibition of peripheral T cell responses in the mammal after the aerosolized antibody administration is less than about 10% of stimulation or inhibition of the peripheral T cell responses that would be detected if the antibody formulation was administered systemically. (Specification: page 11, lines 14-18).

VI. GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

The sole issue on appeal is:

Whether Claims 1, 2, 9-32 and 34-46 are unpatentable under 35 U.S.C. § 103(a) over Lobb et al. (U.S. Patent No. 5,871,734) as evidenced by Arrhenius et al. (U.S. Patent No. 5,869,448) in view of Schramm et al. (*Amer. J. Respir. Cell Mol. Biol.*, **22**(2):218-25 (2000)), Wigzell et al. (U.S. Patent No. 5,958,410) and Krause et al. (U.S. Patent Application Publication No. 2002/0037286).

VII. ARGUMENT

Claims 1, 2, 9-32 and 34-46 have been at least twice rejected under 35 U.S.C. § 103(a) over Lobb et al. (U.S. Patent No. 5,871,734) as evidenced by Arrhenius et al. (U.S. Patent No. 5,869,448) in view of Schramm et al. (*Amer. J. Respir. Cell Mol. Biol.*, 22(2):218-25 (2000)), Wigzell et al. (U.S. Patent No. 5,958,410) and Krause et al. (U.S. Patent Application Publication No. 2002/0037286).

The rejection reasons that it would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to create the claimed invention because Lobb et al. teach aerosol administration of an antibody that binds T cells to treat asthma and Schramm et al. teach that intravenous (i.v.) administration of a different antibody that binds T cells (anti-TCR αβ) can be used to treat asthma. Motivation is alleged because Lobb et al. teach that the antibody can be administered in a variety of art known routes including aerosol. Motivation is further alleged on the basis that Krause et al. teach that antibodies that inhibit T cell activation are preferably administered by pulmonary aerosol and Wigzell et al. teach that pathologic T cells found in the lungs can be treated via intrapulmonary administration of anti-TCR antibody. It is further reasoned that a neutralizing antibody would be used in the claimed method because Schramm et al. teach that asthma symptoms are reduced in the absence of TCRαβ T cells. It is reasoned that with respect to the particular recited dosages of formulation or dosage per weight, a "routineer" would initially test a wide variety of different dosages in order to determine the smallest effective dosage, that the antibody would be administered by a "routineer in conjunction with art known treatments for asthma, and that the antibody would have been administered either before or during asthma symptoms. Finally, the rejection reasons that Lobb et al. teach that the effect can be achieved without detectable blood levels of antibody wherein the aerosol administered antibody would therefore not substantially effect peripheral immune T cell responses. See, *e.g.,* Office Action mailed April 6, 2007 (April 6 Office Action), page 5, which is repeated on page 6.

With respect to the individual references, the rejection further reasons that Lobb et al. teach the use of antibody against VLA-4 to treat asthma, and Arrhenius et al. is cited as teaching that VLA-4 is a receptor on T cells. Lobb et al. is further cited for the following teachings: airway hyperresponsiveness occurs in asthma; the use of humanized anti-VLA-4 antibody and a monovalent

antibody; the anti-VLA4 antibody does not stimulate T cell activation (via an alleged teaching that the antibodies inhibit VLA-4 function); the use of antibody dosages encompassed in the instant Claims 18 and 19; administration of antibody by a nebulized spray; the method of instant Claim 27; the methods of instant Claims 28, 31 and 32; "Lobb et al. teach that the effect seen can be achieved without detectable blood levels of antibody (see column 12, last paragraph) wherein the antibody would not therefore substantially effect peripheral immune function (e.g. because it was not present in the blood)"; the use of the method in humans; and that the method resulted in a 70% decrease in inhibition of late phrase response. The rejection also states that "Lobb et al. disclose: 'For instance, to the extent that the beneficial effects reported herein are due to the inhibition of leukocyte recruitment to VCAM-1 expressing endothelium...' (column 8, last paragraph):, in support of the argument that Lobb et al. contemplate that their method involves inhibition of leukocytes including T cells. The rejection acknowledges that Lobb et al. does not teach the use of anti-TCRαβ antibodies. Schramm et al. is cited for an alleged teaching of the use of intravenous anti-TCR antibodies to treat asthma. The rejection argues that there is no teaching in Schramm et al. that a complete systemic depletion of an entire T cell subset form an animal is required in the antibody treated animals. Krause et al. is cited as teaching that antibodies that inhibit T cell activation are preferably administered by pulmonary aerosol. Wigzell et al. is cited as teaching that pathologic T cells found in the lungs can be treated via intrapulmonary administration of anti-TCR antibody.

To establish a *prima facie* case of obviousness: (1) First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings; (2) Second, there must be a reasonable expectation of success; (3) Third, the prior art reference (or references when combined) must teach or suggest all the claim limitations. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, and not based on applicant's disclosure. *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991). See also MPEP § 2143-§2143.03. An Examiner has the initial burden of establishing a *prima facie* case of obviousness before the burden shifts to the applicant to show otherwise. See, e.g., In re Fine, 5 U.S.P.Q.2d 1596, 1598 (Fed. Cir. 1988). In determining obviousness, one must focus on

Applicant's invention as a whole. *Symbol Technologies Inc. v. Opticon Inc.*, 19 U.S.P.Q.2d 1241, 1246 (Fed. Cir. 1991). The primary inquiry is:

> "whether the prior art would have suggested to one of ordinary skill in the art that this process should be carried out and would have had a reasonable likelihood of success . . . . Both the suggestion and the expectation of success must be found in the prior art, not in the applicant's disclosure." *In re Dow Chemical*, 5 U.S.P.Q.2d 1529, 1531 (Fed. Cir. 1988).

Based on the required showing set forth by the Federal Circuit, Applicant contends that a *prima facie* case of obviousness has not been established in the present case. Arguments are presented below for each claim or group of claims. Claims argued under different subheadings below do not stand or fall together.

<u>Claims 1, 9-15, 17-18, and 24-35</u>

Appellant submits that the combination of references fails to teach or suggest the use of an *aerosolized antibody* having one of the <u>particularly recited receptor specificities</u> (*i.e.,* $\alpha\beta$ TCR, $\gamma\delta$ TCR, CD3, CD4 or CD8) to *reduce airway hyperresponsiveness*, wherein the binding of the antibody to the receptor *causes the depletion or inactivation of the T cell* and wherein the administration of the aerosolized antibody formulation *affects pulmonary T cell responses in the mammal, while peripheral T cell responses in the mammal are neither substantially stimulated nor substantially inhibited.* It is also Appellant's position that the combination of references fails to provide the requisite motivation to combine the references to arrive at the claimed invention and further, fails to provide a reasonable expectation of success to arrive at the claimed invention as claimed in Claims 1, 9-15, 17-18, and 24-35.

The rejection is primarily based on the combination of Lobb et al. and Schramm et al., where the rejection asserts that Lobb et al. teach aerosol administration of an antibody that binds T cells to treat asthma and Schramm et al. teach that a different antibody that binds T cells (anti-TCR$\alpha\beta$) can be used to treat asthma. However, Appellant submits that this combination, even when combined with the other references of Krause et al., Arrhenius et al. and Wigzell et al., fail to teach or suggest the invention as claimed in Claims 1, 9-15, 17-18, and 24-35. Furthermore, there is no

suggestion or motivation found in the references themselves or in the art at the time of the invention to make the combination as the rejection has done. It is the rejection's apparent position that it would be obvious to substitute the antibody of Schramm et al. into a method of Lobb et al., and that based on the teachings of Wigzell et al., Krause et al. and Schramm et al., one of skill in the art would be motivated to do so and would expect success in making the substitution. However, Appellant submits that there is no teaching, suggestion or motivation provided by any of the cited references to substitute the anti-VLA-4 antibody of Lobb et al. with the anti-TCRαβ antibody of Schramm et al., or *vice versa*, even when combined with the other three references. Indeed, neither of Lobb et al. or Schramm et al. attempts to extend its teachings beyond the specific antibody having a specific specificity, described in the reference.

Obviousness cannot be established by combining the teachings of the prior art to produce the claimed invention, absent some teaching or suggesting supporting the combination. ACS Hospital Systems v. Montofiore Hospital, 221 USPQ 929, 933 (Fed.Cir. 1974). "A statement that modifications of the prior art to meet the claim limitations would have been 'well within the ordinary skill of the art at the time the invention was made', because the cited references relied upon teach that all aspects of the claimed invention were individually known in the art is not sufficient to establish a *prima facie* case of obviousness without some objective reason to combine the teachings of the references. *Ex parte Levengood*, 28 USPQ2d 1300 (Bd. Pat. App. & Inter. 1993)." MPEP 2143.01.

With these standards in mind, first, Appellant reviews the rejection's allegation that Lobb et al. teach aerosol administration of an antibody that binds T cells to treat asthma, and the use of this alleged teaching as a basis for including Lobb et al. in the combination of cited references. Lobb et al. is directed to the use of antibodies recognizing VLA-4 integrin, which is a cell adhesion receptor that binds to adhesion molecules such as VCAM-1 (see Arrhenius et al., col. 1, line 54 to col. 2, line 11). Appellant agrees with the rejection that Arrhenius et al. teaches that VLA-4 is a receptor found on T cells, which is all that Arrhenius is alleged to contribute to the combination of references. However, Appellant further notes, as supported by Arrhenius et al. (col. 1, lines 54-65), and by Lobb et al. (col. 2, lines 60-62), anti-VLA4 binds to *a variety of cell types* in addition to T cells, including B lymphocytes, natural killer cells, monoctyes, basophils and eosinophils. This

teaching is relevant to the interpretation of the teachings of Lobb et al., as discussed below. Claim 1 of the present invention is directed to the use of aerosolized antibodies that selectively bind to a receptor on a T cell selected from: an αβ T cell antigen receptor (TCR), a γδ TCR, CD3, CD4 and CD8 (which are all *T cell-specific* receptors), to reduce airway hyperresponsiveness in a mammal.

One important aspect of the rejection reasons that because Lobb et al. teach that anti-VLA4 "treats asthma" in a mammal, and because VLA4 is found on T cells, Lobb et al. accordingly provide a teaching sufficient to combine this reference with a second reference (Schramm et al.) that describes a *different* antibody that binds to a *different* receptor on T cells, expecting that such different antibody will also be useful in the method of Lobb et al. First, it is Appellant's position that one of skill in the art reviewing Lobb et al. would not conclude that the effects on airway hyperresponsiveness observed in Lobb et al. are due to an action of the antibody on T cells, since that conclusion is not presented in Lobb et al. "In determining the propriety of the Patent Office case for obviousness in the first instance, it is necessary to ascertain whether or not the reference teachings would appear to be sufficient for one of ordinary skill in the relevant art having the reference before him to make the proposed substitution, combination, or other modification." *In re Linter*, 458 F.2d 1013, 1016, 173 USPQ 560, 562 (CCPA 1972).

Appellant submits that there is absolutely no teaching or even a suggestion in Lobb et al. that the effects of the anti-VLA4 on airway hyperresponsiveness or any other aspect of asthma, *regardless* of whether the antibody can bind to T cells, are due to any action of the antibody <u>on T cells</u> (*i.e.,* T lymphocytes). Initially, it is noted that a variety of cell types, including basophils, eosinophils, lymphocytes, neutrophils, and monocytes, including macrophages, can be generically referred to as leukocytes or white blood cells, leukocytes being the general term used in Lobb et al. Lymphocytes are a specific subset of leukocytes and include T and B lymphocytes. It is Appellant's position that Lobb et al. teach that the observed effects of anti-VLA4 was on <u>neutrophil and eosinophil</u> recruitment. Indeed, the data of Lobb et al. do not indicate that anti-VLA4 had *any* effect on <u>lymphocyte</u> numbers or recruitment, and upon review of Fig. 4 of Lobb et al., it appears as though anti-VLA4 actually *increased* the lymphocytes in the lungs of the animal (Fig. 4B of Lobb et al.). Lobb et al. provide no other relevant discussion of lymphocytes and no specific mention of T lymphocytes in the patent. However, Lobb et al. provide a *clear teaching* that anti-VLA4

administration caused a significant inhibition of the recruitment of neutrophils and eosinophils to the lung (column 3, lines 4-7; col. 8, line 64 to col. 9, line 2 (note in particular the reference to blocking interactions with *endothelial* cell receptor molecules); Figure 4, and column 12, lines 10-21), which are the only leukocytes to which Lobb et al. appear to attribute the inhibition of the various observed responses. Lobb et al. specifically teach that binding of anti-VLA-4 to leukocytes (*e.g.*, eosinophils and neutrophils) inhibits the migration of such cells to VCAM-1 expressing endothelium, and propose that "antibodies that interfere with the adhesion between leukocyte antigens and *endothelial cell* receptor molecules may be advantageous" (emphasis added) (col. 8, line 63 to col. 9, line 2). Again, as discussed above, Lobb et al., as supported by Arrhenius et al., teach that VLA-4 is present on a variety of different cell types, including eosinophils, which are important mediators of inflammation in asthma. Accordingly, at a minimum, the effects of anti-VLA-4 appear from Lobb et al. to be largely attributable to the action of the antibody on neutrophils and eosinophils, and there is no teaching or suggestion in Lobb et al., explicit or implicit, that the anti-VLA-4 antibody depleted or inactivated T lymphocytes, or that an effect on T cells contributed at all to the observed responses after administration of the antibody.

In contrast, the antibody of the present invention, which binds to a T cell-specific receptor, removes (depletes) and/or inactivates a small and relevant population of T cells which are directly involved in the allergic inflammatory response in the lung. Lobb et al. do not teach or suggest any antibody other than one that binds to VLA-4 or LFA3, which are both adhesion molecules, nor any other mechanism of inhibiting allergic inflammation other than inhibiting the migration of leukocytes (specifically, neutrophils and eosinophils) to lung tissue. Therefore, Lobb et al. can not provide motivation to switch to a different antibody or different mechanism of action, including the one taught by Schramm et al., even when viewed with Wigzell et al. and Krause et al.

Accordingly, it is Appellant's position that Lobb et al. do not teach an aerosolized antibody that binds to a T cell receptor and causes the depletion or inactivation of the T cell, nor do Lobb et al. teach that one should modulate T cells to treat asthma, nor would the teachings of Lobb et al. motivate one of skill in the art to look at the modulation of T cells to treat asthma or airway hyperresponsiveness. At best, the teachings of Lobb et al. would suggest that one should look at methods of targeting *eosinophils or neutrophils* to treat asthma, and could further suggest that

modulation of T cells is not necessary, or is not effective using an anti-VLA4 antibody. This is a *teaching away* from the present invention. Indeed, the only connection between the anti-VLA4 and an action on T cells appears to come from the rejection, and not the teachings of Lobb et al., and would therefore appear to be based on the teachings of the instant specification. The rejection therefore appears to use hindsight in making the obviousness rejection in that the rejection attempts to find each element of the pending claims in the prior art, and then reasons that it is easy to reassemble these elements into the invention; however "it is impermissible to use the claimed invention as an instruction manual or "template" to piece together the teaching of the prior art so that the claimed invention is rendered obvious." *In re Fritch*, 972 F.2d 1260, 1266, 23 USPQ2d 1780, 1784 (Fed. Cir. 1992). The invention must be viewed not with the blueprint drawn by the inventor, but in the state of the art that existed at the time." *Interconnect Planning Corp. v. Feil*, 774 F2d 1132,1138, 227 USPQ 543,547 (Fed. Cir. 1985).

Appellant's position and the teachings of Lobb et al. directly rebut the rejection's position that Lobb et al. provide any teaching or motivation to make the combination with Schramm et al., Wigzell et al., and/or Krause et al. The rejection's position is clearly not a reasonable conclusion based on the Lobb et al. disclosure and would not be the conclusion of one of skill in the art with the reference of Lobb et al. before him.

Appellant further submits that the VLA-4 integrin of Lobb et al. is a completely different cell surface molecule than the recited T cell-specific receptors, such that even if one considers binding of such antibody to a T cell, antibodies that bind to VLA-4 will be expected to have different effects on a T cell than antibodies that bind to one of the T cell receptors recited in the instant claims. For example, anti-VLA4, as taught by Lobb et al. and discussed above, can reduce recruitment of cells to a tissue. In contrast, the recited T cell receptor antibodies are directed against T cell activation receptors and coreceptors, and are depleting or inactivating antibodies (*e.g.,* blocking antibodies are not included). However, the rejection appears to base motivation for combining Lobb et al., Schramm et al., Krause et al. and Wigzell et al. at least in part on the argument that if one antibody that binds to T cells reduces airway hyperresponsiveness, then all antibodies that bind to T cells will reduce airway hyperresponsiveness, regardless of the target protein or mechanism of action, and

further, that results with aerosolized antibodies can necessarily be extrapolated from results using systemically administered antibodies, and *vice versa.*

Using this rationale, one of skill in the art should therefore expect that an antibody that binds to LFA-3 would reduce airway hyperresponsiveness, since LFA-3 is another adhesion protein, like VLA4, that is known in the art to be expressed on T cells, among other cells. However, Lobb et al. demonstrates that anti-LFA3 <u>did not reduce airway hyperreponsiveness</u> when administered to an animal. Referring to Example 2 of Lobb et al. (col. 12, line 37 to col. 13, line 7), where aerosolized anti-VLA-4 administration (antibody HP1/2) was compared to aerosolized anti-LFA-3 administration (antibody IE6), Lobb et al. clearly teaches that <u>administration of aerosolized anti-LFA-3 had no effect on airway hyperresponsiveness</u>. Therefore, the argument that one could simply substitute a different antibody into the specific anti-VLA4 method of Lobb et al. is not correct and is clearly rebutted in the references of record.

Furthermore, Appellant submits that the previously submitted publication by Fahy et al. (enclosed in Evidence Appendix) <u>rebuts</u> the rejection's line of reasoning that it would be expected that a systemically delivered antibody of Schramm et al. would be useful in the method of aerosolized delivery of Lobb et al., because Fahy et al. shows that provision of a therapeutic effect by administration of antibodies <u>systemically</u> does <u>not</u> necessarily mean that the same effect will be provided when the same antibody is administered by aerosol. As discussed on page 5, lines 10-16, Fahy et al. used aerosolized anti-IgE to test whether direct delivery of the antibody to the airway would have the same effect as the systemic delivery of the antibody, which had already been shown to attenuate early and late phase responses to inhaled allergen (Fahy et al., 1999, *Am. J. Respir. Crit. Care Med.* **160**:1023-1027). Fahy's experiment demonstrated that the aerosolized anti-IgE <u>did not</u> attenuate the airway responses to inhaled allergen and in at least one subject, the antibody proved to be immunogenic. Therefore, this experiment shows that, based on the art, one <u>cannot</u> assume that achievement of a therapeutic effect by administration of an antibody systemically can be extrapolated to aerosol administration of the same antibody. The argument can be taken a step further in that the rejection has attempted in the present combination of references to compare two *completely different* antibodies on this basis (*i.e.,* antibodies with different antigen specificities), which makes prediction of effects even more unreasonable. Therefore, the rejection's argument that

one can take the results of Lobb et al. and thereby predict a result using the antibody of Schramm et al. is not fairly based on scientific evidence. Moreover, the teachings of Wigzell et al. and Krause et al. do not contradict the findings of Fahy et al., as neither of Wigzell et al. or Krause et al. had any actual demonstration of the administration of an antibody in aerosol form (discussed in more detail below).

The rejection reasons on page 9 of the April 6 Office Action that: "Fahy et al. hypothesize that the antibody might have been more immunogenic via the aerosol route, but the successful results of Lobb et al. would tend to disagree with this hypothesis", which is speculation put forth by the rejection itself that is not supported by evidence. As discussed above, Lobb et al. investigated the effects of an antibody with a completely different antigen specificity (VLA-4 versus IgE) on airway hyperresponsiveness and provided results that are in contrast to Fahy et al., illustrating the unpredictability of aerosol administration of antibody. The rejection further reasons on page 9 of the April 6 Office Action that the most logical explanation for the results in Fahy et al. "is that their antibody was not effective was because it was antibody that bound a soluble antigen (IgE) present in large quantities in the vascular space wherein said IgE acted as a 'sink of IgE'", and concludes that the results of Fahy et al. are not germane to the claimed invention. Appellants do not see how Lobb et al., directed to a different antigen specificity than that claimed is relevant to the claimed invention, while Fahy et al. is somehow not germane to the invention. Moreover, as discussed above, Lobb et al. teach that aerosol delivery of LFA-3, a protein of the same general type as VLA-4, did not inhibit airway hyperresponsiveness. Based on the reasoning in the rejection, including the rationale for the dismissal of Fahy et al. as being irrelevant, the aerosol administration of LFA-3 would have been predicted to inhibit airway hyperresponsiveness; however, it did not.

Given that Appellant finds no basis in Lobb et al. by way of a teaching, suggestion or motivation for making the combination with Schramm et al. and/or the other cited references, Appellant now reviews the rejection's contention that Schramm et al. teach that anti-TCRαβ can be used to treat asthma, which is the rejection's stated basis for the combination of Schramm et al. with Lobb et al. Appellant submits that Schramm et al. do not teach that anti-TCRαβ can be or should be used *to treat asthma*, and more particularly, Appellant submits that Schramm et al. do not teach that *any antibody*, including anti-TCRαβ, can be used to reduce *airway hyperresponsiveness*, which

is the subject of the instant claims. As defined in the present specification on page 14, lines 7-9, "'airway hyperresponsiveness' or 'AHR' refers to an abnormality of the airways that allows them to narrow too easily and/or too much in response to a stimulus capable of inducing airflow limitation". Appellant emphasizes that this is the subject of the claimed invention (*i.e.,* the reduction of airway hyperresponsiveness in a mammal). In contrast, the only teaching of Schramm et al. related to antibodies is a teaching that the *systemic depletion* in an animal of αβ T cells using anti-TCRαβ, or the *systemic depletion* of γδ T cells using anti-γδ, significantly <u>reduces eosinophils</u>, and to a lesser extent, lymphocytes and macrophages, in bronchoalveolar lavage fluid (BALF) (see Figure 1). These are the *only* experiments in Schramm et al. that use antibodies; the remaining experiments are performed in *TCR knockout mice*. With respect to airway hyperresponsiveness, which is the subject of the instant claims, Schramm et al. do <u>not</u> teach that *any* antibody administered by *any* route can reduce airway hyperresponsiveness, nor do Schramm et al. determine the effect of complete αβTCR depletion on airway hyperresponsiveness using the knockout mice. The only experiments directed to airway hyperresponsiveness in Schramm et al. use wild-type mice or TCR δ-/- mice (*i.e.,* TCR δ knockout mice). Schramm et al. specifically state on page 222, col. 2, last sentence of top paragraph "Methacholine responses were not studied in TCRβ -/- mice...".

Moreover, it is Appellant's position that Schramm et al. lacks any teaching or suggestion to use any antibodies for the treatment of asthma. Schramm et al. teaches that anti-αβTCR, administered *systemically*, reduces the accumulation of various cells in BALF, but it is Appellant's position that this is not a teaching that such an antibody could or should be used to treat asthma. Schramm et al. is a research publication that is primarily directed to determining the role of γδ T cells in asthma, and also to dissect the roles of the two T cell subsets (αβ and γδ). Appellant submits that Schramm et al. <u>do not</u> teach or suggest the therapeutic use of *any* antibodies for the treatment of asthma. In general, Appellant does not find any teaching in Schramm et al. regarding how asthma should be treated. Indeed, complete, systemic depletion of T cells, or substantial depletion of T cells, which is the only use of the antibodies described in Schramm et al., would *not* be viewed by one of skill in the art as a therapeutic approach to treatment of a disease, including airway inflammation and/or hyperresponsiveness, because complete, systemic depletion of a major arm of the immune system as a therapy would have clear, undesirable consequences for the animal.

On page 7 of the April 6 Office Action, the rejection argues that "there is no teaching in Schramm et al. that a complete systemic depletion of an entire T cell subset from an animal is required in the antibody treated animals". First, the only experiments described in Schramm et al. pertain to the use of either TCR knockout mice (*i.e.,* there is a "complete" deletion of T cell subset to which the knockout is directed - see, *e.g.,* page 219, col. 1, first paragraph) and the use of anti-TCR antibodies that deplete the animal of the relevant T cells (see, *e.g.,* page 220, col. 2, last paragraph; "Similar findings were observed in mice depleted of TCRγδ or TCRαβ cells by treatment with monoclonal antibodies (Figure 1)"). Therefore, even if the antibodies do not *completely* deplete the mice of the relevant T cells, clearly, the intent of Schramm et al. is to compare the knockout results to antibody depletion; whether or not complete depletion is achieved via antibody administration. Referring to Figure 1 of Schramm et al., the antibody depletion of T cells is significant, and it is Appellant's position that systemic depletion of T cells, whether complete or substantial, would *not* be viewed by one of skill in the art as a therapeutic approach to treatment of a disease.

Second, and perhaps more relevant to the issue at hand, Appellants refer to the discussion above and again emphasize that *in the antibody-treated animals*, Schramm et al. <u>do not evaluate airway hyperresponsiveness</u>; only eosinophil and other cell accumulation in BALF is evaluated (see Figure 1 of Schramm et al.). Moreover, even *when* airway hyperresponsiveness is evaluated, this is only done in the *γδ TCR knockout mice,* and not in the αβ TCR knockout mice (see page 222 of Schramm et al., "Methacholine responses were not studied in TCRβ-\- mice because every animal studied failed to mount an inflammatory immune response to OVA"). Therefore, there is no teaching in Schramm et al. of the effects of the depletion of T cells having an αβ TCR (complete or partial), by any means, on airway hyperresponsiveness. Accordingly Schramm et al. simply does not teach the use of any antibody against any T cell protein to inhibit airway hyperresponsiveness.

Moreover, Schramm et al. do not teach or suggest the use of aerosolized antibodies or the administration of antibodies to the lung of an animal. One does not learn from the teachings of Schramm et al. that one could or should therapeutically deplete or inactivate the pulmonary T cells in an animal to treat airway hyperresponsiveness in the animal, and moreover, one can not learn from the teachings of Schramm et al. that one can deplete pulmonary T cells and treat airway

hyperresponsiveness without substantially affecting peripheral T cells in the animal. Schramm et al. is not at all concerned with therapeutic approaches to reducing airway hyperresponsiveness, nor to any other T cell-expressed proteins, such as the VLA-4 integrin of Lobb et al. Thus, there is no teaching, suggestion, motivation or expectation of success provided by Schramm et al. to make or use the present invention, even when combined with Lobb et al., alone or in combination with the other references. Accordingly, it is submitted that the rejection's basis for the combination of Schramm et al. with Lobb et al. fails, since Appellant finds no teaching or suggestion in Schramm et al. teach that anti-TCRαβ, including aerosolized anti-TCRαβ, nor anti-TCRγδ, including aerosolized anti-TCRγδ, should be used to treat asthma or specifically, to reduce airway hyperresponsiveness.

Having provided arguments against the rejection's reasoning for combining Lobb et al. and Schramm et al., Appellant now addresses the rejection's stated rationale for the inclusion of Wigzell et al. and Krause et al. in the combination.

The rejection contends that Wigzell et al. provide motivation to combine Lobb et al. and Schramm et al. by teaching that pathologic T cells found in the lungs can be treated via intrapulmonary administration of anti-TCR antibody. However, it is submitted that Wigzell et al. is unrelated to the teachings of either of Lobb et al. or Schramm et al. and do not provide any motivation to combine these two references. Wigzell et al. characterizes T cells from the lungs of patients with <u>sarcoidosis</u>, which is an inflammatory disease in which granulomas form on various tissues and organs, including the lung, lymph nodes, skin, and eyes (col. 1, lines 18-21). Therefore, sarcoidosis, and its treatment, are not limited to the lungs. Wigzell et al. identify a particular subset of T cell receptors that appear to be increased in patients with sarcoidosis, and suggest making an antibody to this particular T cell receptor to treat this specific disease. Appellant submits that sarcoidosis is not related to asthma or airway hyperresponsiveness, nor is the identification of the particular T cell subset by Wigzell et al. relevant to asthma or airway hyperresponsiveness, and therefore, there is no teaching, suggestion or motivation in Wigzell et al. to that would cause one of skill in the art to include this reference in the cited combination or in particular, to combine Lobb et al. with Schramm et al. The rejection emphasizes that Wigzell et al. teach intrapulmonary administration of TCR antibody as a basis for motivation. However, intrapulmonary administration

is listed among a larger group of "known routes" (col. 13, lines 22-25), and there is nothing in this generic teaching that would lead one of skill in the art reading Wigzell et al. to combine the references of Lobb et al. and Schramm et al. Indeed, since Lobb et al. teach aerosolized administration of anti-VLA4, it is not clear how a teaching by Wigzell et al. that antibodies can be administered by intrapulmonary routes provides any information at all, and particularly, with respect to the combination of Lobb et al. with Schramm et al. Moreover, there is no demonstration of the actual administration *in vivo* of any antibody in any form in Wigzell et al., including by aerosol, and so regardless of whether or not Wigzell et al. list various possible routes of administration for antibodies, one of skill in the art has absolutely no expectation that aerosol administration of the Wigzell et al. antibody to treat *any* disease, including sarcoidosis, would be successful. Appellant has provided examples above of instances in which systemic administration did not equate to pulmonary administration, and in which results with one antibody did not equate to results with a different antibody. It is Appellant's position that Wigzell et al. is devoid of any teaching that would remedy the deficiencies of the combination of Lobb et al. and Schramm et al. as discussed above, even in view of Krause et al. and/or Arrhenius et al.

Similarly, the rejection asserts that Krause et al. teach that antibodies that inhibit T cell activation are preferably administered via pulmonary aerosol, which allegedly provides motivation to combine Lobb et al. and Schramm et al. Krause et al. teaches the identification of a protein associated with actin cytoskeletal reorganization called "Fyb/SLAP", which, as with the VLA4 of Lobb et al., is not T cell-specific (the protein is expressed also by macrophages, platelets, and perhaps other hematopoietic cells). Krause et al. teach that one may produce an antibody that selectively binds to Fyb/SLAP and administer the antibody to an animal to regulate cytoskeletal reorganization in the animal. In teaching administration of the therapeutics of their invention, Krause teach a variety of routes, similar to Wigzell et al, although Krause et al. states a preference for pulmonary aerosol delivery for the Fyb/SLAP antibodies. However, Appellant submits that regulation of cytoskeletal reorganization is not related to asthma or airway hyperresponsiveness, nor is the antibody described by Krause et al. specific for T cells, and therefore, there is no teaching, suggestion or motivation in Krause et al. to combine this reference with Lobb et al. and/or Schramm et al. and/or Wigzell et al. Furthermore, the antibody of Krause et al. is not T cell-specific and is

a blocking antibody (see section 0008, 0104, 0105), which is not an antibody that binds to any of the recited T cell receptors and is not an antibody that depletes or inactivates T cells, further removing this reference from any relevance to the claimed invention, even when combined with the other references. Finally, since Lobb et al. teach aerosolized administration of anti-VLA4, it is not clear how a teaching by Krause et al. that antibodies can be administered by pulmonary aerosol routes provides any information at all, and particularly, with respect to the combination of Lobb et al. with Schramm et al. As with Wigzell et al., Krause et al. provide no actual demonstration of the administration of any antibody in any form, including by aerosol, and so regardless of whether or not Krause et al. teach that one route of administration is intrapulmonary, one of skill in the art has absolutely no expectation that aerosol administration of the Krause et al. antibody to treat *any* disease would be successful. It is Appellant's position that Krause et al. is devoid of any teaching that would remedy the deficiencies of the combination of Lobb et al. and Schramm et al. as discussed above, even in view of Wigzell et al. and/or Arrhenius et al.

As will be recognized, claims cannot be found obvious unless the prior art teaches or suggests making the claimed product or process and that there is a reasonable expectation of success at doing so. *See In re Vaeck*, 20 USPQ2d 1438 (Fed. Cir., 1991) (The teaching or suggestion to make the claimed combination or modification and the reasonable expectation of success must both be found in the prior art).

In summary, Lobb et al. does not teach or suggest that anti-VLA4 reduces airway hyperresponsiveness by acting on T cells, but instead teaches the use of anti-VLA-4 to inhibit migration of neutrophils and eosinophils (without any showing of an effect on T cells, other than an *increase* in lymphocytes), and further, Lobb et al. additionally teaches that aerosol administration of another antibody that can bind to T cells (among other cells), anti-LFA3, has no effect on airway hyperresponsiveness. Accordingly, this reference can not provide any teaching, suggestion, motivation or expectation of success at using a T cell-specific antibody that is directed to a completely different antigen and that operates by a completely different mechanism for the treatment of asthma or particularly, airway hyperresponsiveness, regardless of the route of administration. Schramm et al. can not remedy the deficiencies of Lobb et al., because Schramm et al. provides absolutely no connection to the anti-integrin VLA4 antibody, and particularly, because Schramm

et al. does not provide any teaching, suggestion, or expectation of success of treating asthma or reducing airway hyperresponsiveness using any antibody that binds to T cells delivered by any route, because there is no teaching or suggestion in Schramm et al. of the use of such an antibody for such a purpose. Wigzell et al. and Krause et al. are not directed to asthma or airway hyperresponsiveness at all, Krause et al. does not describe a T cell-specific antibody, and neither reference provides any demonstration that an antibody administered by any route will have any therapeutic effect on any disease. Accordingly, the combination of references fails to provide sufficient teaching, suggestion, motivation, or expectation of success at arriving at the presently claimed invention.

Finally, Appellant submits that the claimed invention provides unexpected and surprising advantages over the prior teachings in the art. First, the claimed method targets pulmonary T cell populations in the absence of any substantial effect on peripheral T cells, which is a large advantage over methods which target T cell responses systemically, since peripheral immune responses (i.e., immune responses outside the localized area of delivery, such as in the spleen or lymph nodes) are neither substantially stimulated nor substantially inhibited. Systemically administered antibodies will target all T cells including developing T cells, whereas the aerosolized antibodies of the present invention primarily target T cells at the effector stage, *i.e.* functionally differentiated T cells. The rejection asserts that Lobb et al. meet this claim limitation by teaching that the effect can be achieved without detectable blood levels of antibody (referring to col. 12, last paragraph). However, Lobb et al. merely state at this section of the patent that there were no detectable blood levels of the antibody in the aerosol treated animals. Lobb et al. does not actually demonstrate whether or not the antibody had any effect on any T lymphocytes in the animals. As discussed above, the only teaching of Lobb et al. regarding lymphocytes at all is with respect to Figure 4, where it is shown that anti-VLA4 increases total lymphocytes in the BALF. Even if one assumes, *arguendo*, that aerosolized anti-VLA4 did not substantially affect peripheral T cells, it remains Appellant's position that all evidence in Lobb et al. points to a teaching of an affect on neutrophils and eosinophils, and not lymphocytes, such that there is no teaching or suggestion to arrive at the claimed invention. Moreover, to the extent that the rejection maintains the position that the teachings of Schramm et al. with respect to the systemic administration of antibodies is relevant to the combination of

references, it is clear that Schramm et al. do not teach or suggest any advantage of aerosolized antibodies and solely contemplate total systemic depletion of T cells.

Furthermore, in contrast to reports of the administration of other aerosolized antibodies (e.g., anti-IgE administration, described by Fahy et al. (1999, *Am. J. Respir. Crit. Care Med.* **160**:1023-1027)), the present inventors have demonstrated through working examples that the claimed method is highly effective at reducing airway hyperresponsiveness. Finally, evidence has been provided in the present specification that targeting T cells that are present at the allergic site by the localized administration method of the present invention reduces allergic inflammation-associated exacerbation of AHR without affecting the adaptive immune system.

It is noted that Claim 1 is currently restricted to the elected species of αβ TCR, although it is Appellant's position that the non-elected species of γδ TCR, CD3, CD4 and CD8 are also patentable over the cited combination of references, should the rejection be applied to other species, for substantially the same reasons provided herein. With respect to γδ TCR, although Schramm et al. also describe anti-γδ TCR, the teachings of Schramm et al. are thoroughly discussed above, and the consideration of anti-γδ TCR does not render Schramm et al. any more relevant to the combination than when anti-αβ TCR is considered alone, since the deficiencies of Schramm et al., as well as the other references, remain the same. Furthermore, is noted that none of the cited references teach or suggest an antibody that binds to CD3, CD4 or CD8.

However, to be clear, Appellant asserts that the restricted species as set forth by the rejection with respect to αβ TCR, γδ TCR, CD3, CD4 and CD8, <u>do not</u> stand or fall together.

In summary, in view of the discussion above, the combination of references fails to teach or suggest the use of aerosolized antibody that binds to and depletes or inactivates the recited T cells receptors, whereby aerosolized administration of said antibodies reduces airway hyperresponsiveness in a mammal in the absence of substantially stimulating or inhibiting peripheral T cell responses. Moreover, the combination fails to provide any motivation to make the combination as the rejection has done, or to motivate one to make and use the present invention. Finally, the combination does not provide any expectation of success at making and using the present invention. Therefore, the rejection has not established a *prima facie* case of obviousness in view of the combination of references. In view of the above arguments, Appellant respectfully

requests the Board to direct the withdrawal of the rejection of Claims 1, 9-15, 17-18, and 24-35 under 35 U.S.C. §103(a).

Claim 2

With respect to Claim 2, Appellant notes that this claim is directed to the elected species of αβTCR, whereas Claim 1 in the group of claims above is directed to all species, including non-elected species that were previously rejoined in the Office Action mailed September 8, 2005, and then subsequently restricted again in the Office Action mailed February 22, 2006. Appellant's arguments against the rejection of Claim 2 under 35 U.S.C. § 103(a) are essentially the same as the arguments presented above in view of Claim 1, although such arguments are in the case of Claim 2 directed exclusively to the elected species. However, in the event that Claim 1 falls as a result of consideration of non-elected species in Claim 1, Appellant expressly submits that Claim 2, as well as dependent Claims 9-15, 17-18, and 24-35, to the extent they depend from Claim 1 with respect to the elected invention of αβTCR as recited in Claim 2, do not stand or fall together with Claim 1.

In view of the above arguments, Appellant respectfully requests the Board to direct the withdrawal of the rejection of Claim 2 under 35 U.S.C. §103(a).

Claim 16 and Claims 19-23

In addition to the arguments set forth above for Claims 1, 9-15, 17-18, and 24-35, it is Appellant's further contention that Claims 16 and Claims 19-23 recite particular features of the claimed invention related to advantages of the invention that are not taught or suggested by the combination of references. In particular, Claims 16 and Claims 19-23 recite the use of very low doses of antibody by aerosol administration, which are not taught or suggested by the combination or references, and which it is submitted would be considered to be surprising at the time of the invention. Claim 16 provides the limitation that the antibody is administered at a dose of between about 5 μg antibody and about 10 μg antibody per milliliter of formulation. Claims 19-23 recite doses of less than 40 μg antibody per kg body weight of the mammal (Claim 19), or less than 1 μg per kg body weight of the mammal (Claim 20), or less than 0.5 μg per kg body weight of the

mammal (Claim 21), or less than 0.1 μg per kg body weight of the mammal (Claim 22), or less than 20 ng per kg body weight of the mammal (Claim 23).

As taught in the specification, prior to the present invention, it was thought that antibodies delivered by aerosol must be administered in high doses to overcome the effects of expected low potency and to successfully reach the target airways (see page 10, lines 22-26). For example, U.S. Patent 6,165,463 (see Evidence Appendix) indicates that antibodies are considered to be "low potency" drugs, and therefore indicates that fairly high concentrations of antibodies (e.g., in the milligram per milliliter range) should be formulated for aerosol delivery. The publication of Fahy et al. has been discussed above. Indeed, the lowest dose of antibody specifically taught by Lobb et al. (see col. 6, lines 58-62) is 50 μg per kg body weight of the mammal. Lobb et al. also teach that one could provide a dose to "maintain a plasma level of antibody in the range from 1-1000 μg/ml" (col. 6, lines 52-54), but does not state what actual doses will achieve this range. It is noted that the rejection contends that Lobb et al. specifically teach the dose of Claim 19 (less than 40μg per kg body weight) in col. 6, but such teaching is not found by Appellant. Lobb et al. also teach that the effect of anti-VLA4 (HP1/2) was dose-dependent, and that with intravenous administration, the dose was ineffective below 0.2 mg/kg (col. 12, lines 25-28). Given the teachings in the art at the time of the invention, one would therefore assume that an even higher dose would be needed if the antibody was delivered by aerosol. Delivery of aerosolized antibody in Lobb et al. was provided at 8mg per sheep. Even assuming a 100 kg sheep, this would still be 80 μg per kg body weight. With regard to Schramm et al., this reference does not teach aerosol administration of antibody. With regard to Wigzell et al. and Krause et al., neither of these references demonstrates the delivery of any antibody by any route of administration and provide no specific direction regarding doses for *aerosol* administration. Given the teachings in the art at the time of the invention, such as Fahy et al., it is submitted that one of skill in the art would not expect efficacy in delivering an antibody at the low doses claimed in Claims 16 and 19-23.

In contrast, the method of the present invention is effective at extremely *low* doses of antibody. Indeed, the method of the present invention achieves efficacy with antibody doses that are believed to be about *1000-fold* or more lower than systemic doses of antibody required to achieve the same effect. Doses of antibody as low as 5 μg per ml, delivered by nebulizer to mice

in a plexiglass chamber, which would actually deliver much smaller doses to the airway of each mouse, were effective at reducing airway hyperresponsiveness.

The rejection reasons that with respect to the particular recited dosages of formulation or dosage per weight, a "routineer" would initially test a wide variety of different dosages in order to determine the smallest effective dosage. However, as discussed above, at the time of the invention, it was not generally thought that aerosol delivery of antibodies was efficient or could be achieved at very low doses.

In view of the above arguments, Appellant respectfully requests the Board to direct the withdrawal of the rejection of Claims 16 and 19-23 under 35 U.S.C. §103(a).

Claim 36

In addition to the arguments set forth above for Claims 1, 9-15, 17-18, and 24-35, it is Appellant's further contention that the cited combination of combination of references fails to teach or suggest the use of an aerosolized antibody having one of the particularly recited receptor specificities to reduce airway hyperresponsiveness, wherein the binding of the antibody to the receptor causes the depletion or inactivation of the T cell and *wherein any stimulation or inhibition of peripheral T cell responses in the mammal after the aerosolized antibody administration is less than about 10% of stimulation or inhibition of the peripheral T cell responses that would be detected if the antibody formulation was administered systemically*, as claimed in Claim 36. The claimed method targets pulmonary T cell populations in the absence of any substantial effect on peripheral T cells, which is a large advantage over methods which target T cell responses systemically, since peripheral immune responses (i.e., immune responses outside the localized area of delivery, such as in the spleen or lymph nodes) are neither substantially stimulated nor substantially inhibited. Systemically administered antibodies will target all T cells including developing T cells, whereas the aerosolized antibodies of the present invention primarily target T cells at the effector stage, *i.e.* functionally differentiated T cells. The specification demonstrates that aerosolized administration of the recited antibody reduces airway hyperresponsiveness in a mammal within the claim limitations of Claim 36 (*e.g.,* see Example 5, where any stimulation or inhibition of peripheral T cell responses in the mammal after the aerosolized antibody administration is less than about 10% of

stimulation or inhibition of the peripheral T cell responses that would be detected if the antibody formulation was administered systemically).

Appellant refers to the arguments set forth in detail with respect to 1, 9-15, 17-18, and 24-35, and further submit that none of the cited references, alone or in combination, teach or suggest the claimed limitation that any stimulation or inhibition of peripheral T cell responses in the mammal after the aerosolized antibody administration is less than about 10% of stimulation or inhibition of the peripheral T cell responses that would be detected if the antibody formulation was administered systemically. The rejection reasons that Lobb et al. teach that "the effect seen can be achieved without detectable blood levels of antibody...wherein the aerosol administered antibody would therefore not substantially effect peripheral immune T cell responses", referring to col. 12, last paragraph).

However, it is Appellant's position that Lobb et al. merely state in their patent that there were no detectable blood levels of the antibody (*i.e.,* referenced as "the drug") in the aerosol treated animals. Lobb et al. does not actually demonstrate whether or not the antibody had any effect on any T lymphocytes or any other cells in the periphery of the animals, and specifically, Lobb et al. does not teach that stimulation or inhibition of peripheral T cell responses in the mammal after the aerosolized antibody administration is less than about 10% of stimulation or inhibition of the peripheral T cell responses that would be detected if the antibody formulation was administered systemically. Lobb et al. does not perform an experiment to evaluate T cells, other than to determine that lymphocyte numbers increase in BALF after anti-VLA4 treatment, and has very little disclosure related to T cells at all, and so one can not conclude that Lobb et al. teach the subject matter of Claim 36. Similarly, no such information or evidence is provided in the teachings of Wigzell et al. or Krause et al., and Schramm et al. solely discloses systemic depletion of T cells. Accordingly, it is submitted that the combination of references fails to teach the limitation disclosed in Claim 36, and furthermore, fails to provide any motivation to modify any of the teachings to arrive at the claimed invention.

In view of the above arguments, Appellant respectfully requests the Board to direct the withdrawal of the rejection of Claim 36 under 35 U.S.C. §103(a).

VIII. <u>CLAIMS APPENDIX</u>

The text of the claims involved in this appeal:

1. A method to reduce airway hyperresponsiveness in a mammal that has, or is at risk of developing, airway hyperresponsiveness, comprising administering to the lungs of said mammal an aerosolized antibody formulation comprising antibodies that selectively bind to a receptor on a T cell selected from the group consisting of: a T cell antigen receptor (TCR) selected from the group consisting of an αβ TCR and a γδ TCR, CD3, CD4 and CD8, wherein the binding of the antibodies to the receptor causes the depletion or inactivation of the T cell, wherein administration of the antibody formulation reduces airway hyperresponsiveness in said mammal; and

wherein the administration of the aerosolized antibody formulation affects pulmonary T cell responses in the mammal, while peripheral T cell responses in the mammal are neither substantially stimulated nor substantially inhibited.

2. The method of Claim 1, wherein said receptor on a T cell is an αβ T cell antigen receptor (TCR).

9. The method of Claim 1, wherein said antibody is a humanized monoclonal antibody.

10. The method of Claim 1, wherein said antibody does not stimulate T cell activation.

11. The method of Claim 1, wherein said antibody is a monovalent antibody.

12. The method of Claim 1, wherein said antibody is a neutralizing antibody.

13. The method of Claim 1, wherein said aerosolized antibody formulation is administered at a dose of less than about 500 μg antibody per milliliter of formulation.

14. The method of Claim 1, wherein said aerosolized antibody formulation is administered at a dose of less than about 100 μg antibody per milliliter of formulation.

15. The method of Claim 1, wherein said aerosolized antibody formulation is administered at a dose of less than about 50 μg antibody per milliliter of formulation.

16. The method of Claim 1, wherein said aerosolized antibody formulation is administered at a dose of between about 5 μg antibody and about 10 μg antibody per milliliter of formulation.

17. The method of Claim 1, wherein said aerosolized antibody formulation comprises less than 35% by weight of said antibody.

18. The method of Claim 1, wherein said aerosolized antibody formulation is administered at a dose of less than about 400 μg x $kilogram^{-1}$ body weight of said mammal.

19. The method of Claim 1, wherein said aerosolized antibody formulation is administered at a dose of less than about 40 μg x $kilogram^{-1}$ body weight of said mammal.

20. The method of Claim 1, wherein said aerosolized antibody formulation is administered at a dose of less than about 1 μg x $kilogram^{-1}$ body weight of said mammal.

21. The method of Claim 1, wherein said aerosolized antibody formulation is administered at a dose of less than about 0.5 μg x $kilogram^{-1}$ body weight of said mammal.

22. The method of Claim 1, wherein said aerosolized antibody formulation is administered at a dose of less than about 0.1 μg x $kilogram^{-1}$ body weight of said mammal.

23. The method of Claim 1, wherein said aerosolized antibody formulation is administered at a dose of less than about 20 ng x $kilogram^{-1}$ body weight of said mammal.

24. The method of Claim 1, wherein said aerosolized antibody formulation comprises a pharmaceutically acceptable carrier.

25. The method of Claim 24, wherein said pharmaceutically acceptable carrier is selected from the group consisting of: a dry, dispersible powder; small capsules; liposomes; and a nebulized spray.

26. The method of Claim 1, wherein said aerosolized antibody formulation is administered to said mammal in conjunction with another agent that supports the treatment of AHR selected from the group consisting of: corticosteroids, (oral, inhaled and injected), β-agonists (long or short acting), leukotriene modifiers (inhibitors or receptor antagonists), antihistamines, phosphodiesterase inhibitors, sodium cromoglycate, nedocrimal, and theophylline.

27. The method of Claim 1, wherein said mammal has been sensitized to an allergen and has been exposed to, or is at risk of being exposed to, an amount of said allergen that is sufficient to induce airway hyperresponsiveness (AHR) in said mammal in the absence of said aerosolized antibody formulation.

28. The method of Claim 1, wherein said aerosolized antibody formulation is administered within a time period of between 48 hours or less prior to exposure to an AHR provoking stimulus that is sufficient to induce AHR, and within 48 hours or less after the detection of the first symptoms of AHR.

29. The method of Claim 1, wherein said aerosolized antibody formulation is administered upon the detection of the first symptoms of acute onset AHR.

30. The method of Claim 1, wherein said aerosolized antibody formulation is administered within 1 hour after the detection of the first symptoms of acute onset AHR.

31. The method of Claim 1, wherein said aerosolized antibody formulation is administered within 12 hours or less prior to exposure to a AHR provoking stimulus that is sufficient to induce acute onset AHR.

32. The method of Claim 1, wherein said aerosolized antibody formulation is administered within 2 hours or less prior to exposure to a AHR provoking stimulus that is sufficient to induce acute onset AHR.

34. The method of Claim 1, wherein administration of said aerosolized antibody formulation reduces the airway hyperresponsiveness of said mammal such that the FEV_1 value of said mammal is improved by at least about 5%.

35. The method of Claim 1, wherein said mammal is a human.

36. A method to reduce airway hyperresponsiveness in a mammal that has, or is at risk of developing, airway hyperresponsiveness, comprising administering to the lungs of said mammal an aerosolized antibody formulation comprising antibodies that selectively bind to a receptor on a T cell selected from the group consisting of: a T cell antigen receptor (TCR) selected from the group consisting of an αβ TCR and a γδ TCR, CD3, CD4 and CD8, wherein the binding of the antibodies to the receptor causes the depletion or inactivation of the T cell, wherein administration of the antibody formulation reduces airway hyperresponsiveness in said mammal; and

wherein any stimulation or inhibition of peripheral T cell responses in the mammal after the aerosolized antibody administration is less than about 10% of stimulation or inhibition of the peripheral T cell responses that would be detected if the antibody formulation was administered systemically.

IX. EVIDENCE APPENDIX

A. Fahy et al., 1999, *Am. J. Respir. Crit. Care Med.* **160**:1023-1027 (Submitted in PTO-1449 filed October 4, 2001; cited as evidence by Applicants in Amendment and Response filed May 13, 2003)

B. U.S. Patent 6,165,463 (Submitted in PTO-1449 filed October 4, 2001; cited as evidence by Applicants in Amendment and Response filed October 14, 2003).

X. RELATED PROCEEDINGS APPENDIX

None.

XI. SIGNATURE OF APPELLANT'S REPRESENTATIVE

Correspondence related to this Appeal Brief should be directed to the undersigned agent, who may also be contacted at (303) 863-9700.

Respectfully submitted,

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Brief Communications

Effect of Aerosolized Anti-IgE (E25) on Airway Responses to Inhaled Allergen in Asthmatic Subjects

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Intravenous administration of a humanized monoclonal antibody of IgE (E25) attenuates the early and late phase response to inhaled allergen in allergic asthmatic subjects. To test whether direct delivery of E25 to the airway might have the same effect, we conducted a randomized, double-blind, three group study in 33 subjects with mild allergic asthma (20 to 46 yr of age, 21 men, $FEV_1 > 70\%$ predicted). The airway responses to aerosolized allergen were determined at baseline, after 2 and 8 wk of once daily treatment with aerosolized placebo (n = 11), aerosolized E25 1 mg (n = 12), or aerosolized E25 10 mg (n = 10), and after 4 wk of treatment withdrawal. We found that E25 was detectable in the serum during aerosol treatment, although serum IgE did not change significantly in any of the three groups during treatment. In addition, both doses of E25 were no more effective than placebo in attenuating the early phase responses to allergen at both times during treatment. Although aerosolized E25 was generally well tolerated, one subject receiving aerosolized E25 10 mg daily was found to have serum IgG and IgA antibodies to E25. We conclude that aerosol administration of an anti-IgE monoclonal antibody does not inhibit the airway responses to inhaled allergen in allergic asthmatic subjects. We speculate that the observed lack of efficacy may be due to the inability of aerosol route of delivery to result in high enough concentrations of E25 in the tissue compartments surrounding IgE effector cells to neutralize IgE arising from local airway and pulmonary sources and IgE arising from the vascular space. Additionally, the aerosol route of delivery of monoclonal antibodies may be more immunogenic than the parenteral route. Fahy JV, Cockcroft DW, Boulet L-P, Wong HH, Deschesnes F, Davis EE, Ruppel J, Su JQ, Adelman DC. Effect of aerosolized anti-IgE (E25) on airway responses to inhaled allergen in asthmatic subjects.

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Recombinant humanized monoclonal antibody-E25 or "E25" is a nonanaphylactogenic anti-IgE antibody that attenuates both the early and late phase responses to inhaled allergen in asthmatic subjects (1, 2). In this study, we examined whether aerosolized R25 attenuates the airway responses to inhaled allergen in allergic asthmatic subjects. To do this, we conducted a randomized, placebo-controlled, parallel group clinical trial of the effects of 8 wk of once daily treatment with aerosolized E25 in two doses (1 mg and 10 mg) on the early and late phase responses to allergen challenge in allergic subjects with mild asthma.

Thirty-three subjects with asthma with $FEV_1 \geq 70\%$ predicted, bronchial hyperactivity to methacholine, serum IgE < 500 IU/mL, a positive skin prick test to aeroallergens (house dust mite, perennial ryegrass, birch, cat pelt, or horse hair) were studied (Table 1 and Figure 1). Exclusion criteria were the use of any corticosteroids or symptoms of an upper or lower respiratory tract infection in the previous 6 wk, and history of tobacco use (any in the past 12 mo and total use ≥ 10 pack-years). The study protocol and consent form was approved by the committees for human research at each participating institution, and each subject provided written informed consent.

Subjects were randomized to E25 1 mg, E25 10 mg, or matching placebo (E25 excipient [150 mM NaCl, 10 mM acetate at pH 5.2]); randomization was stratified according to whether the subject had a late phase response to allergen during the screening phase. Subjects self-administered medication once daily at home using a PARI IS-2 nebulizer powered by a PARI Master compressor. On the basis of data from aerosol experiments in monkeys (3), the E25 1 mg dose was predicted to deliver 150 μg to the lower airways and the E25 10 mg dose was predicted to deliver 1.5 mg.

Skin reactivity to house dust mite (*Dermatophagoides pter-*

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TABLE 1

CLINICAL CHARACTERISTICS OF THE STUDY SUBJECTS*

Characteristics	Placebo (n = 9)	E25-1 mg (n = 12)	E25-10 mg (n = 10)
Age, yr	28 ± 8	28 ± 8	30
Weight, kg	72 ± 16	73 ± 20	88 ± 21
FEV_1, % pred	81 ± 14	83 ± 20	84 ± 14
PC_{20}, mg/ml†	0.8	1.4	1.1
	(0.3–2.4)	(0.7–2.6)	(0.5–2.5)
IgE, IU/L	208 ± 171	250 ± 141	226 ± 153

* Plus-minus values are means ± SD.

† PC_{20} denotes the concentration of methacholine that causes a 20% fall in FEV_1. Values are presented as the geometric mean (95% CI).

onynisinus and *Dermatophagoides farinae*), cat pelt, ryegrass (*Lolium perenne*), birch (*Betula* spp), and histamine (1.8 mg/ml), all from Bayer Pharmaceuticals (Spokane, WA) was assessed, as previously described (1, 2).

Bronchial reactivity to methacholine (4) and allergen (1) was determined as previously described. Allergen challenges during the treatment and follow-up phases were performed similarly to baseline, except that the first allergen concentration was two doubling doses below the allergen concentration causing a 20% fall in FEV_1 at baseline. During the treatment and follow-up phases, the allergen challenge continued until the FEV_1 fell by ⩾ 20% or until the same allergen concentration given at baseline was delivered, whichever occurred first.

Total IgE in serum was measured using a microparticle enzyme immunoassay (Abbott Laboratories, Abbott Park, IL). Total IgE in BAL was measured using a more sensitive ELISA (lower limit of detection, 10 pg/ml) as follows: 96-well plates coated overnight with 100 ng of monoclonal anti-IgE antibody in carbonate buffer at pH 9.6 were washed and 100 µl of sample were added. The captured IgE was detected with goat anti-human IgE-biotin (Kirkegaard and Perry, Gaithersburg, MD) and streptavidin-β-galactosidase (Boehringer Mannheim, Indianapolis, IN) followed by 4-methylumbelliferyl-β-D-galactoside substrate (Sigma Chemical Co., St. Louis, MO). The reaction was stopped with 0.3 M glycine at pH 10.5. The fluorescence was read using 360 nm excitation and 460 nm emission wavelength. Free IgE (IgE not in a complex with E25), total E25, free E25, and IgG anti-E25 Fab antibody were measured in blood by ELISA, as previously described (1). Total E25 in serum and BAL samples was measured by ELISA as previously described (1). IgA and IgM class anti-E25 antibodies in blood were assayed similarly using plates coated overnight at 4° C with 300 ng of E25 Fab fragment in 100 µl of PBS. The plates were washed with 0.05% Tween 20 in PBS, then incubated with assay diluent (0.5% BSA/0.05% Tween 20/0.01% thimerosal in PBS) for 1 h to block nonspecific binding sites. Samples were diluted 1/100 in assay diluent, either with added excipient control or with 100 µg/ml E25. The plates were washed and the diluted samples were incubated in the wells for 1 h. The plates were washed and either goat anti-

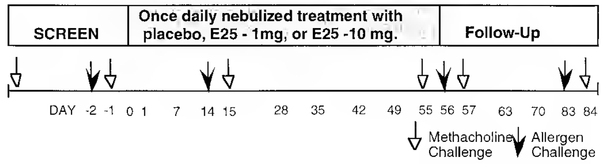

Figure 1. Diagrammatic representation of the three-phrase, 14- to 16-wk, randomized, double-blind, placebo-controlled, parallel group trial. There was a 2- to 4-wk screening phase, followed by an 8-wk treatment phase, followed by a 4-wk follow-up phase. During the screening phase, subjects were characterized by spirometry, methacholine reactivity, and allergen skin tests, and they were taught to record their peak flows in a diary using a Mini-Wright Peak Flow Meter (Clement Clarke, Columbus, OH). Subjects then underwent baseline methacholine challenge and airway allergen challenge in the sequence depicted in the diagram, after which they began self-administering study drug (E25-1 mg, E25-10 mg, or matched placebo) once a day by nebulizer for 8 wk. Methacholine challenge and allergen challenge were repeated as shown in the diagram. Spirometry was performed on Days 0, 14, 28, 42, 56, 70, and 84. Samples of venous blood for standard tests of hematologic, renal, and hepatic function were collected once during the screening phase and again on Days 0, 14, 28, 56, and 83. Samples of venous blood for levels of free IgE, total IgE, and rhuMAb-E25 were collected at Days 0, 14, 28, 42, 56, 70, and 83. Finally, bronchoscopy and bronchoalveolar lavage was performed on the 10 subjects enrolled at the Québec center during the screening phase and again on Day 42.

TABLE 2

PULMONARY FUNCTION AT BASELINE, AT THE END OF TREATMENT, AND DURING FOLLOW-UP*

Outcome	Study Group	Day 0	Day 14	Day 56	Day 83
FEV_1, L	Placebo (n = 9)	3.1 ± 0.6	3.1 ± 0.6	3.2 ± 0.6	3.2 ± 0.6
	E25-1 mg (n = 12)	3.4 ± 1.1	3.6 ± 1.1	3.7 ± 1.1	3.6 ± 1.1
	E25-10 mg (n = 10)	3.4 ± 0.9	3.5 ± 0.9	3.8 ± 0.8	3.6 ± 0.9
	Study Group	Week −1	Week 2	Week 8	Week 12
AM PEF, L/min†	Placebo (n = 9)	456 ± 99	460 ± 84	452 ± 98	463 ± 108
	E25-1 mg (n = 12)	494 ± 96	493 ± 88	498 ± 99	498 ± 102
	E25-10 mg (n = 10)	532 ± 78	527 ± 79	529 ± 75	515 ± 89
	Study Group	Day −1	Day 15	Day 57	Day 84
PC_{20}, mg/ml	Placebo (n = 8)	0.95	0.77	1.17	1.0
		(0.33–2.73)	(0.30–2.00)	(0.38–3.61)	(0.41–2.42)
	E25-1 mg (n = 9)	0.92	1.39	1.33	1.31
		(0.40–2.14)	(0.61–3.16)	(0.43–4.13)	(0.58–2.98)
	E25-10 mg (n = 10)	1.07	0.82	1.41	1.19
		(0.48–2.38)	(0.40–1.70)	(0.53–3.74)	(0.55–2.56)

* Plus-minus values are means ± SD; PC_{20} values are presented as the geometric mean (95% CI).

† AM PEF refers to peak expiratory flow rates measured in the morning before bronchodilator medication was taken.

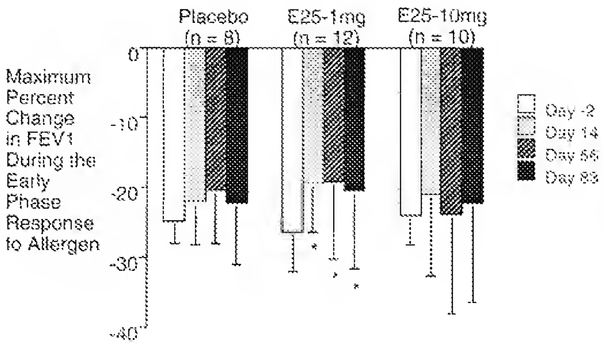

Figure 2. Effect of aerosolized E25 on the change in FEV_1 observed during the first hour after allergen challenge (early phase response) in the three treatment groups for each of the four allergen challenges performed during the study (*see* Figure 1). Data are presented as mean ± SD. *Asterisks* denote significantly different from Day −2, but not significantly different from the change seen in the placebo group).

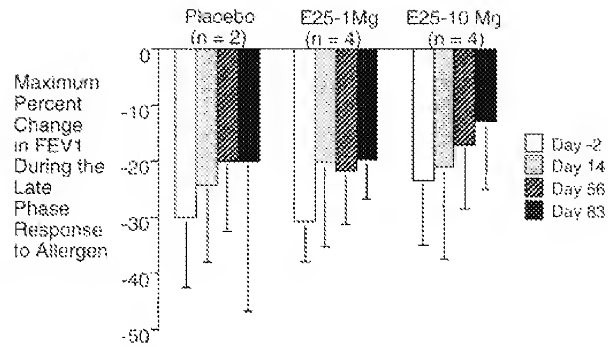

Figure 3. Effect of aerosolized E25 on the change in FEV_1 observed between 3 and 7 h after allergen challenge (late asthmatic response) in the subgroup of subjects in each treatment group who demonstrated a significant late phase response during the screening phase. Data are presented as mean ± SD.

human IgA-HRP conjugate (American Qualex) diluted 1/1,000 or goat antihuman IgM-HRP conjugate (Sigma) diluted 1,500 was added. After incubation in the wells for 1 h, the plates were washed and the color was developed using OPD.

Total E25 and IgE concentrations in BAL fluid were corrected for dilution using a urea-based dilution factor. Urea was measured in the BAL using a blood urea nitrogen end point assay kit (Sigma) and a modified protocol for sensitive urea detection (5).

Bronchoscopy was performed as previously described (6). For bronchoalveolar lavage (BAL) three 50 ml boluses of 0.9% saline solution at 37° C were instilled and then aspirated. The BAL fluid was centrifuged at 2 to 8° C for 10 min; 2 ml of the supernatant was aliquoted and diluted with 2 ml of the BAL diluent (1% BSA/0.1% Tween 20/20 mM phosphate/0.9% NaCl). The diluted BAL sample was filtered through a 0.22 µM centrifugal filter (Millipore Ultrafree MC; Millipore Corp., Bedford, MA) at 4,000 × *g* for 5 min.

Data are described as mean and standard deviation or as the geometric mean with 95% confidence intervals, as appropriate. Values for peak flow were analyzed by calculating weekly averages from daily data collections. The area under the curve (AUC) for allergen-induced changes in FEV_1 during the early (0 to 1 h) and late (3 to 7 h) phases was calculated using the trapezoidal rule (percent fall in FEV_1 × minutes). Between- and within-group comparisons were made using Wilcoxon's rank sum and signed-rank tests, respectively. A p value ≤ 0.05, using two-tailed tests, was considered statistically significant.

Thirty-one of the 33 enrolled subjects completed the study. One subject in the placebo arm developed an asthma exacerbation on Day 7 of treatment, and another subject in the placebo arm wihdrew consent on Day 11 because of a change in living location. One additional subject in the placebo group was unable to undergo methacholine on Days 55 and 57 or allergen challenge on Day 56 because of acute labyrinthitis. Overall, aerosolized E25 was well tolerated (Table 2). There were no serious adverse events during the study, and there were no statistically significant differences among treatment groups in the incidence of adverse events. However, whereas only three of 11 subjects in the placebo group reported headache, nine of 12 subjects in the 1 mg group and eight of 10 subjects in the 10 mg group reported headache during the treatment phase.

Compliance was assessed by counting used and unused medication vials at each study visit during the treatment phase. Using this measure of compliance, we found that nine of the 12 subjects in the placebo group, 11 of the 12 subjects in the 1 mg group, and nine of the 10 subjects in the 10 mg group completed at least 90% of all 56 treatments.

Serum total IgE levels (free IgE + IgE complexed to E25) did not change significantly during the treatment phase, and serum-free IgE concentrations were similarly unaffected (data not shown). Changes in concentrations of serum total or free IgE were not expected because of the low serum concentra-

TABLE 3

EFFECTS OF E25 AND PLACEBO ON EARLY AND LATE PHASE AIRWAY RESPONSES ASSESSED AS AREA UNDER THE CURVE FOR CHANGE IN FEV_1 DURING ALLERGEN CHALLENGE*

Outcome	Study Group	Day 0	Day 14	Day 56	Day 83
Early response	Placebo (n = 8)	−980 ± 186	−910 ± 424	−801 ± 338	−874 ± 407
	E25-1 mg (n = 12)	−1,058 ± 310	−767 ± 395	−783 ± 363	−842 ± 527
	E25-10 mg (n = 10)	−925 ± 320	−797 ± 431	−884 ± 653	−880 ± 653
Late response	Placebo (n = 2)	−3,675 ± 2,221	−3,130 ± 959	−3,000 ± 3,088	−4,500 ± 8,545
	E25-1 mg (n = 4)	−5,483 ± 752	−2,948 ± 2,553	−3,593 ± 2,715	−2,400 ± 2,100
	E25-10 mg (n = 4)	−3,855 ± 2,172	−2,835 ± 2,957	−1,665 ± 1,693	−1,695 ± 2,405

* Plus-minus values are means ± SD. The area under the curve (AUC) for allergen-induced changes in FEV_1 during the early (0 to 1 h) and late (3 to 7 h) phases was calculated using the trapezoidal rule (percent fall in FEV_1 × minutes).

TABLE 4

NUMBER OF SUBJECTS WITH DETECTABLE SERUM E25 CONCENTRATIONS*

Group	Day 0	Day 14	Day 28	Day 42	Day 56	Day 70	Day 83
Placebo	1/11 (28.0)	0/11 (ND)	0/11 (ND)	0/11 (ND)	0/11 (ND)	0/11 (ND)	0/11 (ND)
E25-1 mg	0/12 (ND)	1/12 (20)	4/12 (23 ± 5)	4/12 (33 ± 11)	3/12 (37 ± 17)	1/12 (35)	1/12 (20)
E25-10 mg	0/10 (ND)	10/10 (73 ± 34)	8/10 (105 ± 50)	6/10 (143 ± 71)	6/10 (143 ± 80)	4/10 (98 ± 61)	3/10 (61 ± 39)

* Values in parentheses represent the mean ± SD E25 level in ng/ml for the subjects in whom a detectable level was found.

tions of E25. Concentrations of serum E25 were insufficient to complex significant amounts of serum IgE.

The concentration of allergen delivered during the treatment period was similar to the concentration delivered at baseline in all the treatment groups. For example, in the E25-10 mg group, the concentration of allergen delivered on Day 56 was 0.10 doubling doses less on average (standard deviation of 0.32 doubling doses) than at baseline; the corresponding doubling dose values for the E25-1 mg group and the placebo were 0.58 (2.07) and 0.25 (0.71), respectively; p = 0.78 between groups. Treatment with E25-1 mg was associated with a significant within-group attenuation in the early phase response to allergen, but this change was not significantly greater than placebo, and the E25-10 mg treatment group did not show any significant within- or between-group effect (Figure 2 and Table 3). Two subjects in the placebo group, four subjects in the E25-1 mg group, and four subjects in the E25-10 mg group had a late phase response to allergen. In this small subgroup of subjects, there was no statistically significant difference in the magnitude of the late phase response during treatment in any group (Figure 3, Table 3).

E25 was undetectable in serum samples from the placebo group except for a single observation on Day 0 (Table 4). Serum levels of E25 were detectable in both the low dose (1 mg) and the high dose (10 mg) groups. In the low dose group, detectable serum levels of E25 were found in four of the 12 subjects at some point during the study period (Table 4). In the high dose group, a larger production of subjects had detectable serum E25 levels, especially during the initial treatment period when all 10 of the subjects had detectable levels (Table 4 and Figure 4). Notably, the frequency with which E25 levels were detectable in the E25-10 mg group declined during the treatment phase from 10/10 to 6/10 (Table 4 and Figure 4). The number of empty medication vials returned by subjects in the high dose group was similar for all treatment visits.

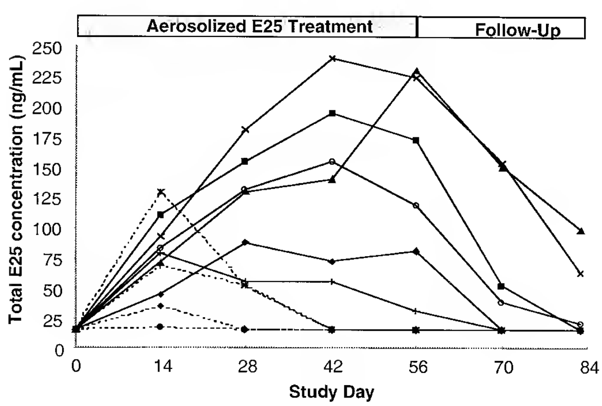

Figure 4. E25 levels in blood from the 10 subjects in the 10 mg group at different time points during the 8-wk treatment period and during the 4-wk follow-up period. Four subjects (*dashed lines*) had E25 levels that were undetectable by Day 42 of treatment despite higher levels earlier in the treatment period.

A serum IgG antibody directed against E25 was detected at Day 28 of treatment in one subject (Subject 0204) in the high dose E25 group (Table 5); antibodies were not detected in any other subject in any other group. This finding coincided with a decline in serum E25 concentration in this subject. The antibody remained detectable at the end of the follow-up period (Day 83), but it was undetectable during an additional special follow-up visit 11 wk after study completion. Clinical examination, chest radiograph, pulmonary function tests (including a test of diffusing capacity), and analysis of blood and urine did not reveal any evidence of immune-complex-mediated disease in this subject 11 wk after study completion.

Four subjects in the 10 mg group had no detectable E25 in blood at Day 56 (Figure 4). Blood samples from these four subjects were extensively studied for anti-E25 antibodies of IgG, IgA, and IgM classes. The subject who had an IgG anti-E25 response also had an IgA anti-E25 response. No significant reactivity of any antibody class was found in the other three subjects.

The levels of E25 in BAL were highly variable (Table 6) but generally within the range expected from data available from a preclinical aerosol study in cynomolgus monkeys (Theresa Sweeney, Ph.D, personal communication). Notably, one of the subjects in the 10 mg group had no detectable E25 in BAL on Day 42. Serum levels of E25 were also undetectable for this subject from Day 28 onward. No positive anti-E25, IgG, IgA, or IgM antibody titers were detected in this subject, and there was no indication of noncompliance. The disappear-

TABLE 5

LEVELS OF E25 AND IgG ANTI-E25 IN SUBJECT 0204 IN THE E25-10 mg GROUP

Day	Free Serum E25 (*ng/ml*)	Total Serum E25* (*ng/ml*)	Anti-E25 Titer†
0	ND	ND	< 2.0
14	ND	35.3	< 2.0
28	ND	ND	2.1
42	ND	ND	2.4
56	ND	ND	2.5
70	ND	ND	2.4
83	ND	ND	2.4
158	Not done	ND	< 2.0

* Refers to E25 complexed to IgE (free E25 refers to E25 not in a complex with IgE).
† Refers to anti-Fab titer. Anti-Fc titer was < 2.0 at all time points.
ND denotes not detected.

TABLE 6

LUNG-DEPOSITED DOSES OF E25, BAL IgE LEVELS, AND E25:IgE RATIOS IN THE SUBGROUP OF 10 SUBJECTS WHO UNDERWENT BAL

Subject No.	Group	Theoretical Deposited Daily Dose (μg)	Estimated Deposited Dose (μg)	BAL Dilution Corrected IgE (μg/ml)	Ratio of BAL E25/BAL IgE
1	Placebo	0	0	NC	NC
2	Placebo	0	0	0.103	NC
3	Placebo	0	0	0.131	NC
4	Placebo	0	0	0.092	NC
5	E25-1 mg	150	170–274	0.045	301
6	E25-1 mg	150	19–31	0.085	18
7	E25-1 mg	150	707–1,140	0.169	336
8	E25-10 mg	1,500	1,470–2,380	0.059	2,000
9	E25-10 mg	1,500	273–440	0.100	221
10	E25-10 mg	1,500	ND	0.058	NC

Definition of abbreviations: NC = not calculated; ND = not detected.

ance of detectable drug levels in the lung and serum may still be due to noncompliance or to anti-E25 antibodies of IgG, IgA, or IgM isotypes not identified by the immunoassays used here. Total IgE concentrations in BAL ranged from 12 to 151 ng/ml and averaged approximately 15% of the serum concentrations of total IgE at screening visit 1 (Table 6). Total IgE concentrations in BAL on Day 42 ranged from 45 to 169 ng/ml and averaged 21% of serum total IgE concentrations. Calculated ratios of E25:IgE from Day 42 BAL samples ranged from 18.3 to 2,000:1 (Table 6).

The main finding of our study is that E25, a nonanaphylactogenic anti-IgE antibody, delivered by the aerosol route was no better than aerosolized placebo in attenuating the airway responses to inhaled allergen in allergic asthmatic subjects. This result is in contrast to previous findings in protocols where E25 was delivered intravenously.

The early phase response to allergen at Weeks 2 and 8 after initiation of treatment with aerosolized E25-1 mg daily was significantly less than the baseline early phase response, but the degree of attenuation was not significantly greater than placebo. The effects of E25-10 mg on the early phase response were no greater than the effects of E25-1 mg and also no greater than placebo. Similarly, analysis of data for the late phase response in the subgroup of subjects who had a significant late phase response at baseline showed no significant attenuation for either the 1 mg or the 10 mg dose groups. These data for the late phase response have to be interpreted very cautiously because of the small number of subjects with a late phrase response at baseline. Previously, in similar protocols using similar methods, we have shown that E25 administered intravenously significantly reduces both the early and late phase responses to inhaled allergen (1, 2). Thus, the aerosol route of delivery for E25 is not as effective as the intravenous route in attenuating airway responses to inhaled allergen.

There are at least three reasons for the lack of efficacy of aerosolized E25 in this study. First, the aerosol route of delivery may not have delivered sufficient E25 to the lower airways. E25 clearly reached the lower airways after aerosolization because E25 was detected in BAL and blood, and any swallowed E25 would have been inactivated in the stomach. However, it is possible that the aerosol route of delivery did not result in high enough concentrations of E25 to neutralize IgE in the lung tissue compartments surrounding IgE effector cells. The vascular space, in particular, represents a large "sink" of IgE constantly available to move into the lung interstitium to replace IgE complexed with E25. A second possible explanation for the lack of efficacy of aerosolized E25 in this study is that neutralizing antibodies to E25 developed and prevented E25 from binding free IgE. Antihuman antibodies have never been detected after intravenous administration of humanized E25 that contains less than 5% murine amino acid. However, the aerosol route of administration of E25 may be more immunogenic than the intravenous or subcutaneous route. A third possible explanation for the lack of efficacy of aerosolized E25 in this study is that the subjects were noncompliant. Compliance appears to have been good in this study, however, as evidenced by the counts of used and unused medication vials at each study visit.

In summary, we found that aerosolized E25 did not significantly attenuate the airway responses to inhaled allergen in asthmatic subjects. One subject developed an IgG and IgA antibody against E25, suggesting that the aerosol route of delivery for monoclonal antibodies may be more immunogenic than the intravenous or subcutaneous route. We conclude that the aerosol route of delivery for E25 is not likely to be a useful treatment for allergic asthma.

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[45] **Date of Patent: Dec. 26, 2000**

[54] **DISPERSIBLE ANTIBODY COMPOSITIONS AND METHODS FOR THEIR PREPARATION AND USE**

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[21] Appl. No.: **09/323,276**

[22] Filed: **Jun. 1, 1999**

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[62] Continuation of application No. 08/951,312, Oct. 16, 1997, which is a continuation-in-part of application No. 08/423,515, filed as application No. PCT/US96/05070, Apr. 12, 1996.

[51] **Int. Cl.**[7] **A61K 39/395**

[52] **U.S. Cl.** **424/130.1**; 514/951

[58] **Field of Search** 514/535, 171, 514/951; 424/130.1

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[57] **ABSTRACT**

This invention relates to dispersible antibody compositions and methods for preparing and using these compositions. In particular, the present invention relates to dry powder dispersible antibody compositions wherein antibody conformation is preserved. The compositions have good powder dispersibility and other desirable characteristics for pulmonary delivery of therapeutic antibodies.

14 Claims, 4 Drawing Sheets

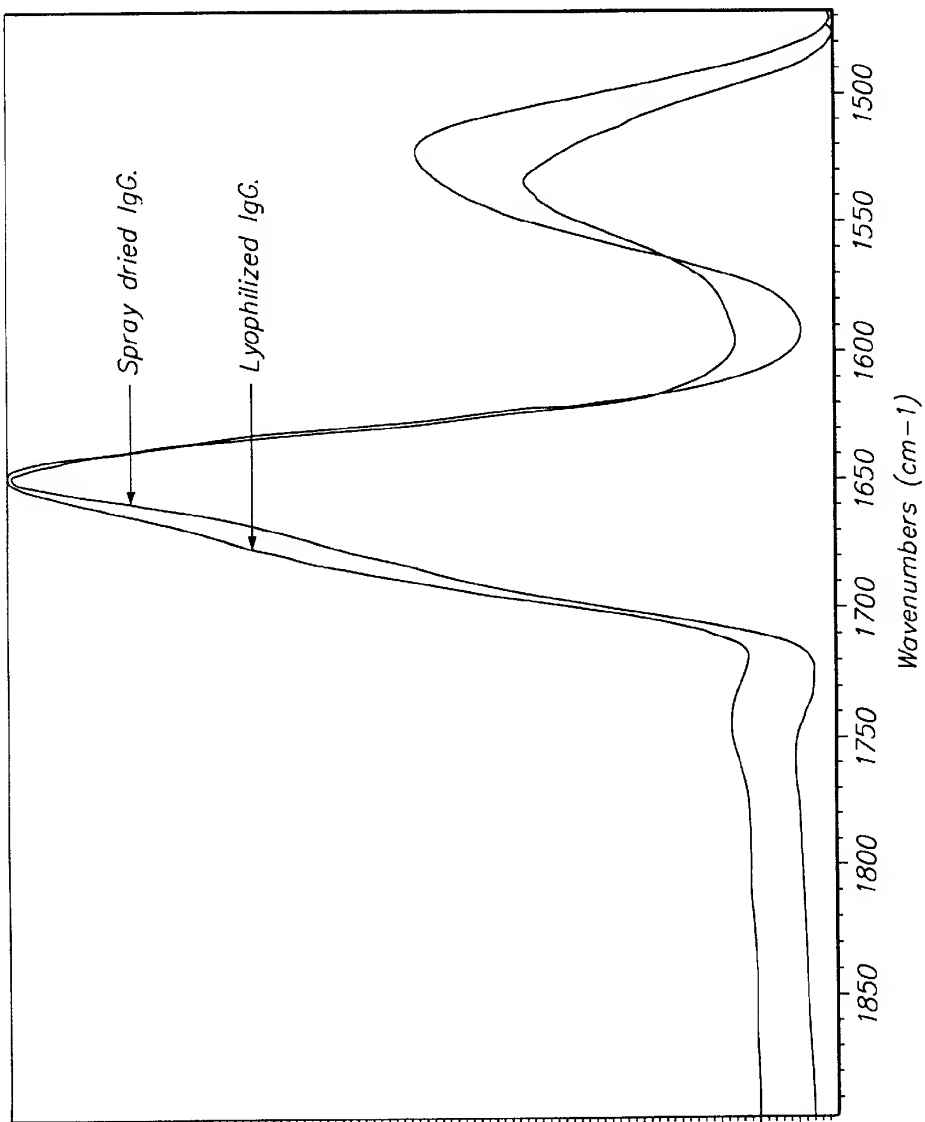
Spray dried IgG.
Lyophilized IgG.
Absorbance
0.036
0.034
0.032
0.030
0.028
0.026
0.024
0.022
0.020
0.018
0.016
0.014
0.012
0.010
0.008
0.006
0.004
0.002
0.000
−0.002
−0.004
1850
1800
1750
1700
1650
1600
1550
1500
Wavenumbers (cm−1)

FIG. 1

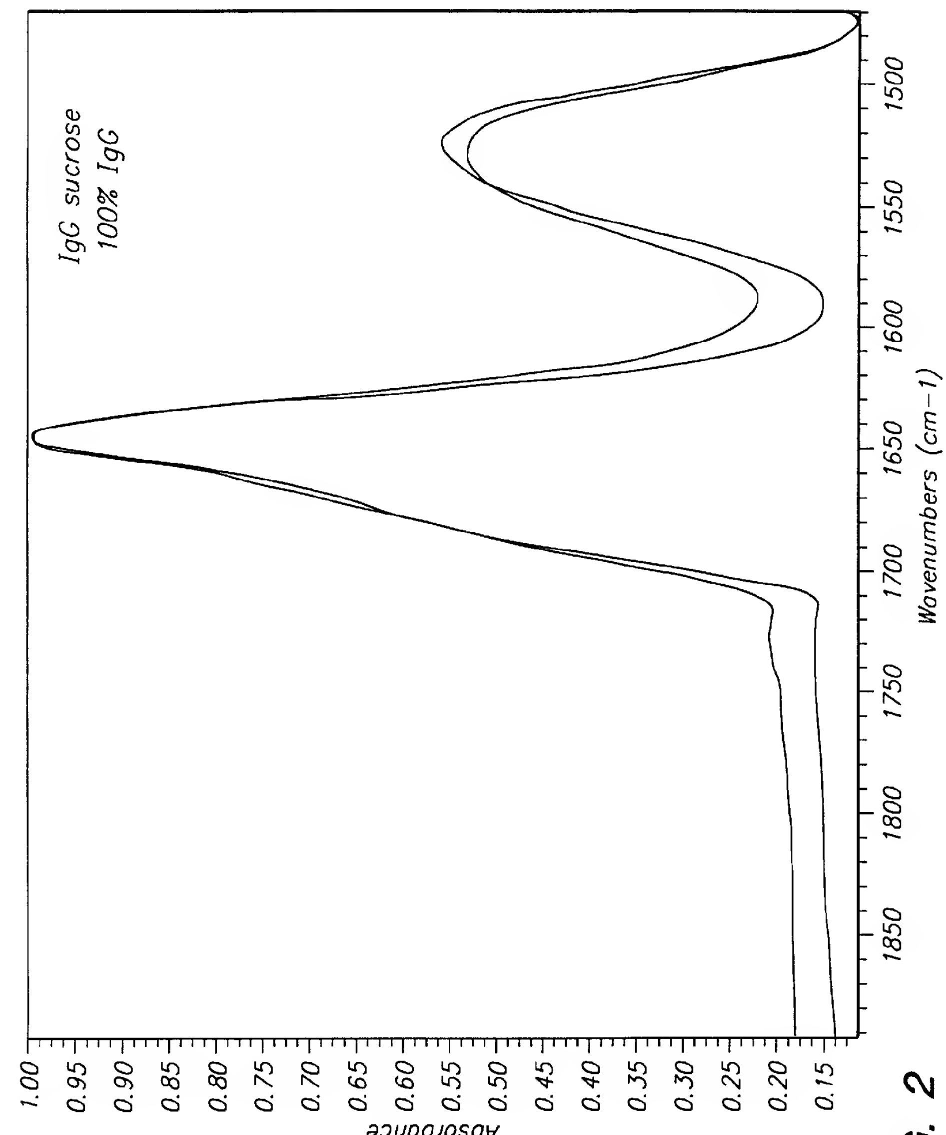
IgG sucrose
100% IgG
Absorbance
1.00
0.95
0.90
0.85
0.80
0.75
0.70
0.65
0.60
0.55
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
Wavenumbers (cm-1)
1850
1800
1750
1700
1650
1600
1550
1500

FIG. 2

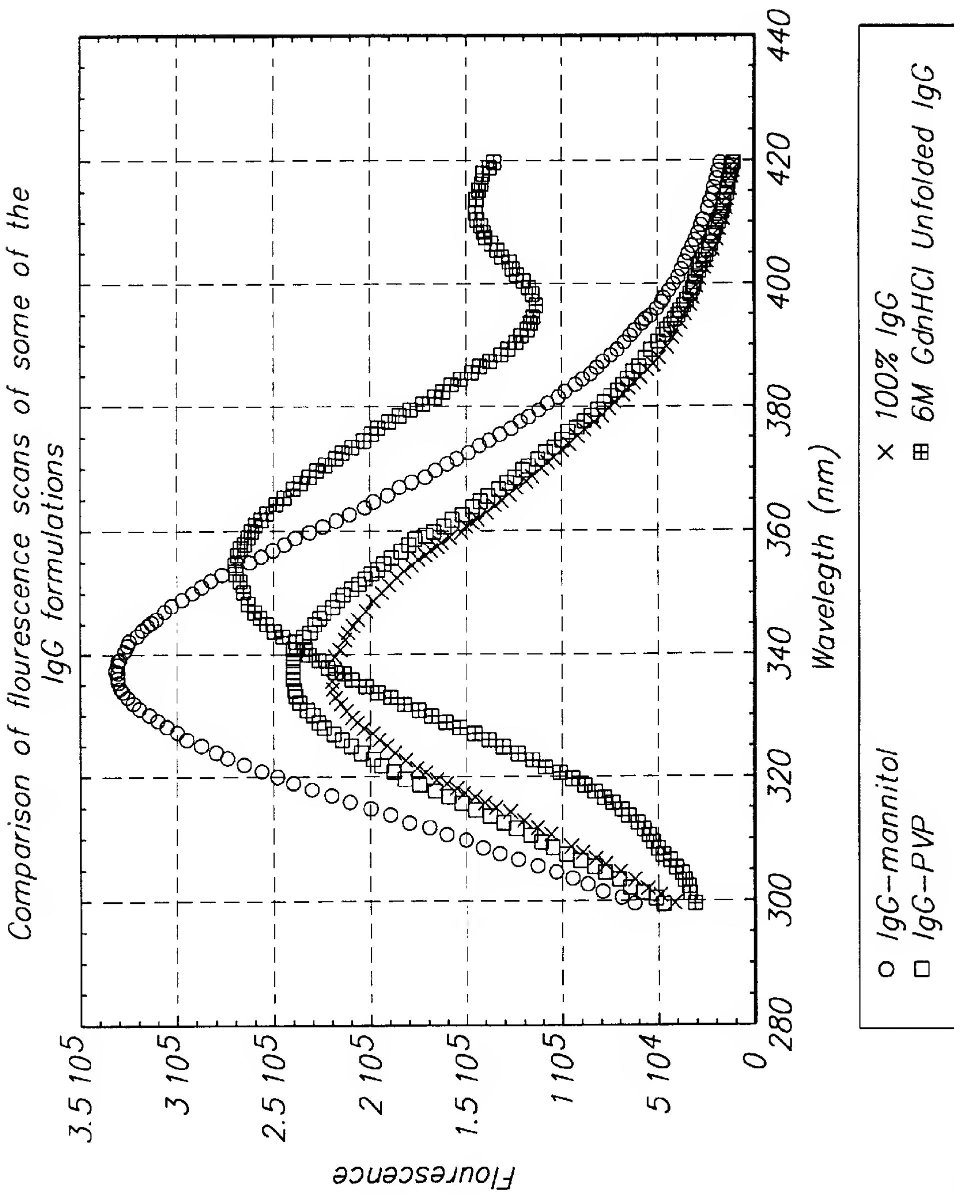
Comparison of flourescence scans of some of the IgG formulations
Flourescence
3.5 10^5
3 10^5
2.5 10^5
2 10^5
1.5 10^5
1 10^5
5 10^4
0
280
300
320
340
360
380
400
420
440
Wavelegth (nm)
IgG--mannitol
IgG--PVP
100% IgG
6M GdnHCl Unfolded IgG

FIG. 3

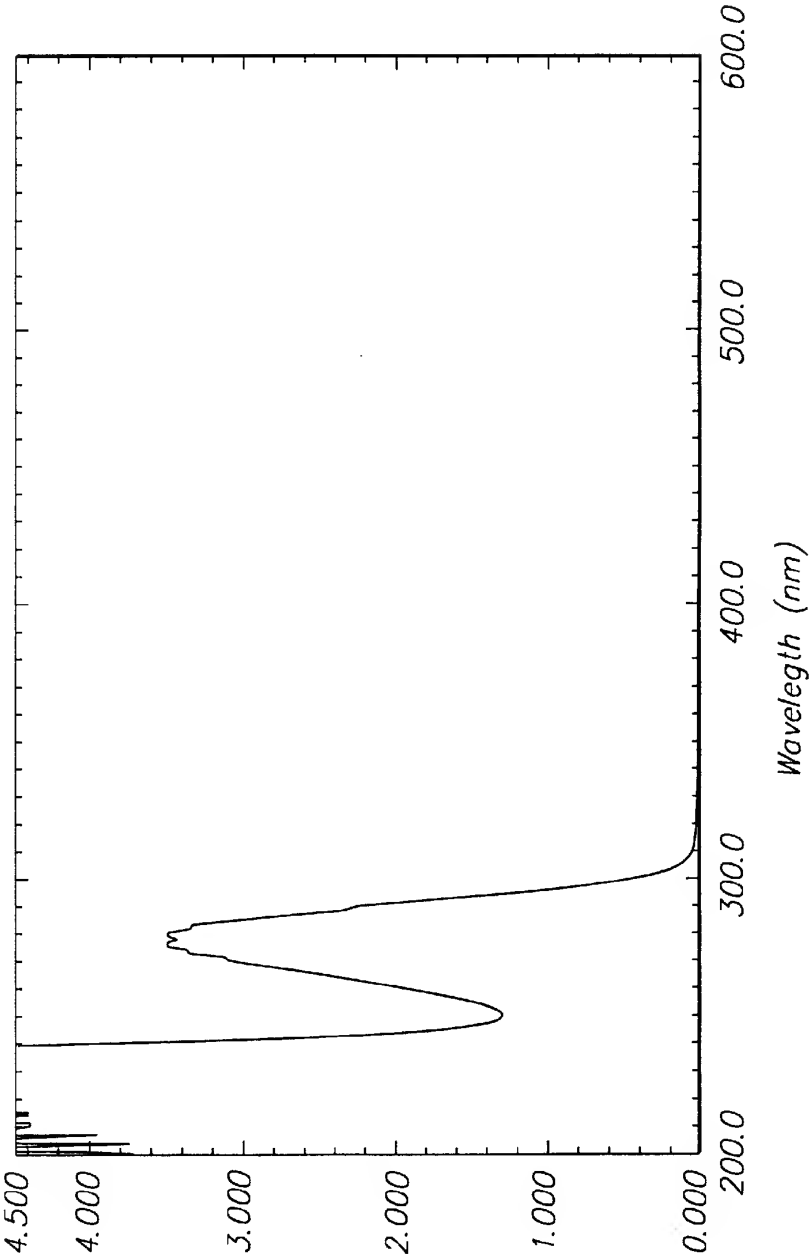
4.500
4.000
3.000
2.000
1.000
0.000
200.0
300.0
400.0
500.0
600.0
Wavelegth (nm)

FIG. 4

DISPERSIBLE ANTIBODY COMPOSITIONS AND METHODS FOR THEIR PREPARATION AND USE

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a continuation of U.S. application Ser. No. 08/951,312 filed Oct. 16, 1997. This application is a continuation-in-part of U.S. application Ser. No. 08/423,515 filed Apr. 14, 1995 and PCT Application No. PCT/US96/05070 filed Apr. 12, 1996, which applications are incorporated by reference herein in their entirety.

FIELD OF THE INVENTION

This invention relates to dispersible antibody compositions and methods for preparing and using these compositions. In particular, the present invention relates to dry powder dispersible antibody compositions wherein antibody conformation is preserved. The compositions have good powder dispersibility and other desirable characteristics for pulmonary delivery of therapeutic antibodies.

BACKGROUND OF THE INVENTION

The protective effects of humoral immunity are known to be mediated by a family of structurally related glycoproteins called antibodies. Antibodies initiate their biological activity by binding to antigens. Antibody binding to antigens, which may be covalent or noncovalent, is exquisitely specific for one antigen and is often very strong.

Antibodies are produced in a membrane-bound form by B-lymphocytes. Blood contains many different antibodies, each derived from a clone of B-cells and each having a distinct structure and specificity for antigen. Antibodies are present in the cytoplasmic compartment and on the surface of B-lymphocytes, in the plasma, in interstitial fluid of the tissues and in secretory fluids such as saliva and mucous. Surfaces of immunoeffector cells, such as mononuclear phagocytes, natural killer cells and mast cells also have antibodies.

All antibodies are similar in their overall structure, accounting for certain similarities in physiochemical features such as charge and solubility. All antibodies have a common core structure of two identical light chains, each about 24 kilodaltons, and two identical heavy chains of about 55–70 kilodaltons each. One light chain is attached to each heavy chain, and the two heavy chains are attached to each other. Both the light and heavy chains contain a series of repeating homologous units, each of about 110 amino acid residues in length which fold independently in a common globular motif, called an immunoglobulin (Ig) domain. The region of an antibody formed by the association of the two heavy chains is hydrophobic. Antibodies, and especially monoclonal antibodies, are known to cleave at the site where the light chain attaches to the heavy chain when they are subjected to adverse physical or chemical conditions. Because antibodies contain numerous cysteine residues, they have many cysteine-cysteine disulfide bonds. All Ig domains contain two layers of beta-pleated sheets with three or four strands of anti-parallel polypeptide chains.

Despite their overall similarity, antibody molecules can be divided into a small number of distinct classes and subclasses based on physiochemical characteristics such as size, charge and solubility, and on their behavior in binding to antigens. In humans, the classes of antibody molecules are: IgA, IgD, IgE, IgG and IgM. Members of each class are said to be of the same isotype. IgA and IgG isotypes are further subdivided into subtypes called IgA_1, IgA_2 and IgG_1, IgG_2, IgG_3 and IgG_4. The heavy chains of all antibodies in an isotype share extensive regions of amino acid sequence identity, but differ from antibodies belonging to other isotypes or subtypes. Heavy chains are designated by the letters of the greek alphabet corresponding to the overall isotype of the antibody, e.g., IgA contains α, IgD contains δ, IgE contains ϵ, IgG contains γ, and IgM contains μ heavy chains. IgG, IgE and IgD circulate as monomers, whereas secreted forms of IgA and IgM are dimers and pentamers, respectively, stabilized by the J chain. Some IgA molecules exist as trimers.

There are 1×10^7, and perhaps as many at 10^9, structurally different antibody molecules in every individual, each with the unique amino acid sequence in their antigen combining sites. Sequence diversity in antibodies is confined to three short stretches within the amino terminal domains of the heavy and light chains. The amino acid sequences of the amino terminal domains are called variable (V) regions, to distinguish them from the more conserved constant (C) regions.

Antibodies have several known therapeutic applications. For example, they may be used to bind to and block cell markers and receptors. Antibodies to microorganisms may be used to inhibit or inactivate the microorganism and/or prevent or treat disease conditions caused by these microorganisms. When antibodies bind to microorganisms, they enhance their recognition and destruction by macrophages. Antibodies may be used as anticytokines, antichemokines, antihormones, antiinflammatories and immunosuppressors or as antineutrophil adhesion agents. Antibodies, especially monoclonal antibodies, may be used systemically to deliver therapy. In these cases antibodies are often used as immunoconjugates, immunoliposomes or immunomicrospheres. Antibodies can be used as apoptosis stimulators and as recognizers of cancerous and precancerous cells. Known examples of antibodies which may be useful therapeutically include the following.

MedImmune Inc. is studying the use of humanized anti-RSV monoclonal antibodies and markets a polyclonal anti-RSV antibody from donor blood (RespiGam) to treat respiratory syncytial virus (RSV) infections. MedImmune also markets CytoGam, an anti-CMV (cytomegalovirus) human immune globulin for the treatment of CMV infection. IDEC and Genentech are jointly performing clinical trials of a chimeric mouse-human monoclonal antibody (rituximab) aimed at the CD20 antigen found on mature B cells and most non-Hodgkin's lymphoma tumors for use in treating relapsed or refractory low-grade non-Hodgkin's lymphoma. GalaGen is studying the use of the polyclonal antibody Diffistat-G for treatment of Clostridium difficile antibiotic associated diarrhea. Smith Kline and Schering Plough are developing an anti-IL-5 antibody which has been shown in clinical trials to prevent eosinophilic inflammation and airway constriction. An anti-IgE antibody is being developed by Genentech to "switch-off" allergies. Monoclonal antibody Rhu-Mab-E25, which is a humanized chimeric IgG_1 monoclonal antibody for a unique epitope on human high affinity IgE receptors (FceRI), has been shown to reduce free IgE levels after the first administration by injection. It attenuated both early and late phase responses to inhaled allergens after multiple injections. Examples of antibodies used therapeutically also include a nebulized IgG (Sandoz), which is used intranasally against respiratory syncytial virus (RSV); HNK20 (Oravax), an anti-RSV IgA; and 4B9

(Bristol Myers-Squibb), an anti-group B Streptococcus IgM monoclonal antibody. Other therapeutically useful antibodies include anti-CD4 antibodies, anti-IL-2 antibodies and anti-IL4 antibodies.

The immunotherapy of respiratory syncytial virus infection using small particle aerosols of IgG has been disclosed by Piazza et al. (The Journal of Infectious Diseases, Vol. 166, pp. 1422–1424, 1992) In this study it was shown that a 15-minute exposure to an aerosolized 5% solution of IgG effected a 50-fold reduction in pulmonary virus. Brown (Aerosol Science and Technology, Vol. 24, pp. 45–56, 1996) discloses the use of antibodies as inhibitors or antagonists of cytokines to depress respiratory inflammatory diseases or allergen-induced asthmatic responses. Also disclosed is local respiratory delivery of pathogen-specific antibody for treatment of acute viral or bacterial respiratory infections. Antibody liposomes, i.e., immunoliposomes, are disclosed by Maruyama et al. in Biochemica et Biophysica Acta, Vol. 1234, pp. 74–80, 1995. Coating liposomes with antibody leads to enhanced uptake of the immunoliposome by the reticuloendothelial system. Human monoclonal antibodies are known to be useful as antitumor agents. A mouse/human monoclonal IgG_1 antibody specific for the Lewis Y antigen found on the surface of tumor cells is disclosed by Paborji et al. (Pharmaceutical Research, Vol. 11, No. 5, pp. 764–771, 1994). The use of antibodies in metered-dose propellant driven aerosols for passive antibody aerosol therapy against respiratory infections is disclosed in Brown et al. (Journal of Immunological Methods, Vol. 176, pp. 203–212, 1994). Immune responses in the respiratory tract are of great importance for protection against infections of the respiratory system and for their involvement in respiratory allergies and asthma. Effective targeting of immunomodulating reagents including antibodies to the respiratory tract is shown to be of benefit in increasing local immunity to respiratory pathogens or decreasing immune mediated respiratory pathology. Inhaled immunoconjugates, immunoliposomes or immunomicrospheres have application in the lung as killers of cancer cells (immunoconjugates) or, in the case of immunoliposomes and microspheres, as stealth delivery particles of a variety of therapeutic agents. An IgM anti-group B Streptococcus monoclonal antibody is disclosed by Gombotz et al. (Pharmaceutical Research, Vol. 11, pp. 624–632, 1994).

Over the years certain drugs have been sold in compositions suitable for forming a drug dispersion for oral inhalation (pulmonary delivery) to treat various conditions in humans. Such pulmonary drug delivery compositions are designed to be delivered by inhalation by the patient of the drug dispersion so that the active drug within the dispersion can reach the lung. It has been found that certain drugs delivered to the lung are readily absorbed by the alveolar region directly into the blood circulation. Pulmonary delivery is particular promising for delivery of macromolecules such as proteins, polypeptides, high molecular weight polysaccharides and nucleic acids, which are difficult to deliver by other routes of administration. Such pulmonary delivery can be effective both for systemic delivery and for localized delivery to treat diseases of the lungs.

Pulmonary drug delivery can itself be achieved by different approaches, including liquid nebulizers, aerosol-based metered-dose inhalers (MDI's) and dry powdered dispersion devices. Chlorofluorocarbon (CFC) based MDI's are losing favor because of their adverse effect on the environment. Dry powder dispersion devices, which do not rely on CFC aerosol technology, are promising for delivering drugs that may be readily formulated as dry powders. Many otherwise labile macromolecules may be stably stored as lyophilized or spray dried powders, either by themselves or in combination with suitable powder carriers.

The ability to deliver pharmaceutical compositions as dry powders, however, is problematic in certain respects. The dosage of many pharmaceutical compositions is often critical, so it is desirable that dry powder delivery system be able to accurately, precisely and reliably deliver the intended amount of drug. Moreover, many pharmaceutical compositions, including antibodies, are quite expensive. Thus, the ability to efficiently formulate, process, package and deliver the dry powders with minimal loss of drug is critical. While the permeability of natural macromolecules in the lung is well known, combined inefficiency of macromolecule production processes and macromolecule delivery has limited commercialization of dry macromolecule powders for pulmonary delivery. It is also essential that dry powders for pulmonary delivery be readily dispersible prior to inhalation by the patient in order to assure adequate distribution and systemic absorption.

An important requirement for hand held and other powder delivery devices is efficiency. It is important that the delivered dose be relatively high to reduce the number of breaths required to achieve a total dosage. The ability to achieve both adequate dispersion and small dispersed volumes is a significant technical challenge that requires in part that each unit dosage of the powder composition be readily and reliably dispersible. Certain pulmonary delivery devices, such as those disclosed in U.S. Pat. No. 5,458,135 and International Patent Publication WO96/09085 are useful for pulmonary delivery of dry powder drugs.

Spray drying is a conventional chemical processing operation used to produce dry particulate solids from a variety of liquid and slurry starting materials. The use of spray drying for the formulation of dry powder pharmaceuticals is known but has usually been limited to small molecule and other stable drugs which are less sensitive to thermal degradation and other rigorous treatment conditions. The use of spray drying for the preparation of biological macromolecule compositions, including antibodies, can be problematic since such macromolecules are often labile and subject to degradation when exposed to high temperatures and other aspects of the spray drying process. Excessive degradation of the macromolecules can lead to drug formulations lacking in the requisite purity.

It can also be difficult to control particle size and particle size distribution in compositions produced by spray drying. For pulmonary delivery it is critical that the average particle size be maintained in a respirable range and that the amount of the composition comprising particles outside the target size range be minimized. Moreover, it can sometimes be difficult to achieve a desired low moisture content required for physical and chemical stability in the final particulate product, particularly in an economic manner. Finally, and perhaps most importantly, it has been difficult to produce the small particles necessary for pulmonary delivery in an efficient manner. For high value macromolecular drugs, high collection efficiencies, i.e., the amount of particulate drug recovered from the process in a usable form, are important. While spray drying has been used to prepare powders of macromolecules in laboratory scale equipment, commercial spray dryers are not designed to produce powders in the pulmonary size range. The methods for atomization, drying powder and collection must be modified to economically produce a protein powder with the desired product characteristics for pulmonary delivery and in sufficient yield and at commercially acceptable production rates, i.e., in excess of

30 grams per hour. Useful methods are disclosed, for example, in International Patent Application No. PCT/US97/07779, the disclosure of which is incorporated herein by reference in its entirety.

SUMMARY OF THE INVENTION

The present invention provides dispersible dry powder antibody compositions and methods for their preparation and use. These dispersible dry powder antibody compositions have a moisture content of less than about 10% by weight (%w) water, usually about 1–5% by weight, and preferably less that about 3% by weight of water. They have a particle size of about 0.1 to 7 μm mass median diameter (MMD), usually about 0.4 to 5 μm MMD, preferably about 1 to 4 μm and most preferably about 1–3 μm MMD. The dispersible dry powder antibody compositions of the present invention result in a delivered dose of greater than about 30%, usually greater than about 40%, preferably greater than about 50% and most preferably greater than about 60%. They have an aerosol particle size distribution of about 1–5 μm mass median aerodynamic diameter (MMAD), usually about 1.5–4.5 μm MMAD and preferably about 1.5–4.0 μm MMAD, or with at least about 40% (preferably at least about 50%) of the particles less than about 3.3 μm in diameter. The compositions contain at least about 40% by weight antibody. Further, the antibodies in these compositions are not aggregated and are present in their native conformation so as to retain their biological activity.

According to the present invention, antibody containing compositions having improved characteristics which overcome at least some of the deficiencies noted above with respect to prior compositions are provided. The present invention provides compositions and methods which provide a predetermined concentration of antibody, and, optionally, other excipients as a dry dispersible powder. The antibody is optionally formulated prior to spray drying with compatible excipients such as sugars, buffers, salts, surfactants, polymers, other proteins and other specific stabilizing additives as needed to provide a therapeutically effective dose, inhibit degradation during drying, promote powder dispersibility and achieve acceptable physical and chemical stability of the powder at room temperature while maintaining antibody integrity.

In one aspect the invention provides dry powder compositions for pulmonary delivery comprising an antibody that is substantially in its native conformation. Such compositions retain antibody activity upon reconstitution.

In another aspect the invention provides antibody-based dry powder compositions which are spray dried from the antibody and, optionally, excipient, in a solvent under conditions to provide a respirable dry powder. Solvents for such mixtures may include water and ethanol.

In a yet further aspect, the invention provides methods for preventing or treating a condition by administering the dry powder antibody compositions described above.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** illustrates comparative FT-IR spectra of spray dried and lyophilized 100% IgG. This figure shows that the spray dried powder retained antibody integrity.

FIG. **2** illustrates comparative FT-IR spectra of 100% IgG and IgG:sucrose:citrate (70:20:10) powders, both of which retained native conformation.

FIG. **3** is a fluorescence scan of some antibody powders of the present invention. No alteration in antibody conformation after spray drying was seen.

FIG. **4** illustrates a UV spectrum of an IgG:mannitol:citrate (70:20:10) powder after reconstitution. No light scattering at 400 nm was seen, indicating the absence of aggregation.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is based at least in part on the discovery that antibodies may be formulated as dispersible dry powder compositions while retaining their integrity. The dispersibility characteristics of the subject antibody based compositions means that they are more suitable for use in pulmonary delivery devices than antibody compositions prepared by other methods. The compositions of the invention are readily aerosolized and rapidly absorbed through the lungs of a host when delivered by a dry powder inhaler. Standard lyophilized antibody formulations do not consist of particles with size suitable for pulmonary delivery. In contrast, the dry powder formulations of the present invention retain antibody conformation and stability, are readily dispersible for pulmonary delivery and allow for unit dose packaging.

The invention consists of compositions comprising antibodies in dry powder dispersible formulations. The use of dry particles of a certain size range allows for delivery of antibodies to the alveolar area of the lungs. Optionally, the dry powder formulations of the present invention may contain stabilizers and excipients such as sugars, polymers and other proteins. The compositions of the present invention are useful in pulmonary dry powder drug delivery systems, including but not limited to those disclosed in U.S. Pat. No. 5,458,135 and International Patent Publication WO96/09085.

A. Definitions

As used herein the following terms have the following meanings:

The terms "dispersibility" or "dispersible" mean a dry powder having a moisture content of less than about 10% by weight (%w) water, usually below about 5%w and preferably less that about 3%w; a particle size of about 0.4 to 5 μm mass median diameter (MMD), usually about 1 to 4 μm MMD and preferably 1–3 μm MMD; a delivered dose of greater than about 30%, usually greater than about 40%, preferably greater than about 50% and most preferably greater than about 60%; and an aerosol particle size distribution of about 1–5 μm mass median aerodynamic diameter (MMAD), usually about 1.5–4.5 μm MMAD and preferable about 1.5–4.0 μm MMAD, or with at least about 40% (preferably at least about 50%) of the particles less than about 3.3 μm in diameter.

The term "non-aggregated" means that the antibody is substantially oligomer-free. This means that usually less than about 15%, preferably less than about 10% and more preferably less than about 5% of the antibody in the composition comprises covalently or non-covalently bound dimers, trimers or larger aggregates.

The term "native conformation" means that the antibody in the composition substantially retains the secondary and tertiary structure of the original state of the molecule.

The term "powder" means a composition that consists of finely dispersed solid particles that are free flowing and capable of being readily dispersed in an inhalation device and subsequently inhaled by a subject so that the particles reach the spaces of the deep lung to permit penetration into the alveoli. Thus, the powder is said to be "respirable."

The term "dry" means that the composition has a moisture content such that the particles are readily dispersible in an

inhalation device to form an aerosol in the absence of a liquid propellant. This moisture content is generally below about 10%w water, usually below about 5%w and preferably less than about 3%w.

The terms "pharmaceutical excipient" or "additive" mean compounds which stabilize antibody and/or improve powder aerosol performance and stability. The types of excipients useful in the present invention include proteins such as human serum albumin (HSA), carbohydrates such as sucrose, alditols such as mannitol, salts such as sodium citrate, polymers such as PVP and Ficoll, and the like.

The term "antibody" means the structurally related glycoproteins which bind to antigens. The term antibody includes monoclonal antibodies and/or IgA, IgD, IgE, IgG and IgM isotype antibodies, including these compounds present in the form of immunoconjugates, immunoliposomes or immunospheres.

B. Compositions:

The present invention is drawn to dispersible antibody-containing dry powder compositions suitable for pulmonary delivery. The compositions comprise a therapeutically effective amount of an antibody, optionally in combination with a pharmaceutically acceptable carrier or excipient.

These dry powder compositions comprise a plurality of discrete, dry particles with an average particle size below about 7 μm, preferably in the range from 0.4–5 μm, more preferably from 1–4 μm and most preferably from 1–μm. The average particle size of the powder is measured as mass mean diameter (MMD). Such powders are capable of being readily dispersed in an inhalation device and subsequently inhaled by a patient so that the particles are able to reach the alveolar regions of the lungs.

A particular characteristic which relates directly to improved dispersibility and handling characteristics of respirable dry powders is rugosity. Rugosity is the ratio of the specific area (measured by molecular surface adsorption or another known technique) and the surface area calculated from particle size distribution (as measured by a centrifugal sedimentary particle size analyzer) and particle density (as measured by pycnometry), assuming non-porous spherical particles. If the particles are known to be generally nodular in shape, as spray dried particles are, rugosity is a measure of the degree of convolution or folding of the surface. A rugosity of 1 indicates that the particle is spherical and non-porous. Rugosity values greater than 1 indicate that the particle surface is non-uniform and convoluted to at least some extent, with higher numbers indicating a higher degree of non-uniformity. For the antibody-based powders of the present invention, it has been found that particles preferably have a rugosity of at least 2, more preferably of at least 3.

Prior dispersible dry powder formulations of macromolecules have shown that certain peptides and proteins could be formulated into dispersible dry powder compositions suitable for pulmonary delivery. However, the molecular weight of the peptides and proteins previously formulated is lower than the molecular weight of the antibodies formulated in the present invention, which have molecular weights of from about 150 to about 400 kilodaltons. It is well known that larger proteins are subject to degradation such as cleavage due to physical stress. Thus, they are not easily formulated into dispersible dry powders suitable for pulmonary delivery. Accordingly, it is unexpected that antibodies can be so formulated, as shown in the present invention.

The moisture content of the dry powder particles of the present invention is usually below about 10% by weight water, preferably below about 5%w and more preferably below about 3%w. Such low moisture content powders are generally physically and chemically stable during storage at room temperature and are readily dispersible in an inhalation device to form an aerosol.

Examples of antibodies which may be formulated using the present invention include those which have biological activity or which may be used to treat a disease or other condition. They include, but are not limited to, antibodies to microorganisms (including respiratory pathogens), monoclonal antibodies directed against tumor antigens and antibodies to cell receptors (including receptors involved in inflammation and allergy). Immunoconjugates of each of these examples may also be formulated using the present invention. Analogs, derivatives, fragments and pharmaceutically acceptable salts of the above may also be used. They may also be formulated with lipids, liposomes, microspheres or the like.

Antibodies suitable for use in the compositions of this invention include IgA, IgE, IgG, IgD and IgM. IgA, IgG and IgM antibodies are preferred, with IgG and IgA antibodies being particularly preferred. The amount of antibody which constitutes a therapeutically effective amount will vary in the composition depending on the biological activity of the antibody employed and the amount needed in the unit dosage form. The condition to be treated or prevented will also determine the amount of antibody required, as will the subject to which the antibody composition is being administered. Because antibodies are generally low potency drugs, the compositions comprise at least about 40% by weight antibody in the formulation, preferably between about 70% to about 100% and most preferably about 70% to about 90%. The amount of excipients and pharmaceutically acceptable carriers may be from about 0–60%, preferably from about 0–30% and most preferably from about 10–30% by weight.

Compositions according to the present invention comprise dispersible antibody powders intended for pulmonary delivery, i.e., inhalation by a patient into the alveolar regions of the patients lungs. The compositions comprise particles having an average particle size below about 10 μm. Preferably the particles of the composition will have a moisture content below about 10% by weight, more preferably below about 5% by weight and typically below about 3% by weight. Preferably at least about 50% by weight of the composition will comprise particles having a particle size less than about 5 μm, more preferably 75% of particles in the range from 0.4 μm to 5 μm. The compositions will often be packaged as unit doses where a therapeutically effective amount of the antibody composition is present in a unit dose receptacle, such as a blister pack, gelatin capsule, or the like, so long as a moisture barrier is provided.

Pharmaceutical excipients and/or additives useful in the present invention include proteins (e.g., HSA, recombinant human albumin (rHA), gelatin and casein), peptides (e.g., aspartame) and amino acids (e.g., alanine, glycine, arginine, glutamic acid and aspartic acid) which improve conformational stability of antibody during spray drying and also improve dispersibility of the powder. Carbohydrates/sugars and alditols are also particularly useful. Suitable carbohydrate/sugar compounds include sucrose, trehalose, lactose, raffinose, and the like. Suitable alditols include mannitol and pyranosyl sorbitol and the like. Polymeric excipients/additives include polyvinylpyrrolidones (PVP), Ficolls, soluble hydroxy ethyl starch, dextrates and the like of suitable molecular weight. Suitable pH adjusters or buffers include organic salts prepared from organic acids and bases, such as sodium citrate, glycine, sodium tartrate, sodium lactate, tromethamine and the like. Also useful are

small amounts of pharmaceutically acceptable surfactants such as Tweens, chelators such as EDTA and inorganic acids and bases such as sodium phosphate and the like. Other suitable pharmaceutical excipients and/or additives include those disclosed in *Pharmaceutical Sciences*, Remington, 18th ed. (1990), the disclosure of which is incorporated herein by reference.

The antibody-based dry powder compositions of the present invention may be produced by spray drying solutions or slurries of the antibody and, optionally, excipients, in a solvent under conditions to provide a respirable dry powder. Solvents may include polar compounds such as water and ethanol, which may be readily dried. Antibody stability may be enhanced by performing the spray drying procedures in the absence of oxygen, such as under a nitrogen blanket or by using nitrogen as the drying gas.

Spray drying IgG with the optimized conditions described in the Examples section produced antibody-containing powders with particle size distribution between 1.2–1.8 μm MMD. The moisture content of these powders ranged from 1.3–3.5% by weight.

DISCLOSURE OF THE EXAMPLES OF THE INVENTION

The following examples are not intended to limit the scope of the invention in any manner.

Materials and Methods:

In general the following materials and methods were used in the examples that follow unless otherwise indicated.

Materials:

IgG, a glyocosylated protein of 150 kilodalton molecular weight purified from pooled normal human serum by fractionation on ion-exchange chromatography was purchased from Sigma. The IgG used was an essentially salt-free (less than 1% sodium), lyophilized bulk substance.

Formulation excipients were research grade or better. Citrate buffer salts used in formulation preparation were USP/ACS grade. The following excipients were used in preparing the IgG powders: Recombinant human serum albumin (Miles); Citric acid, monohydrate (JT Baker); Trisodium citrate, dihydrate (YT Baker); sucrose (EM Science); mannitol (USP); polyvinylpyrrolidone (PVP) k-15, molecular weight 10 kilodaltons (ISP Tech) and Ficoll (Pharmacia), a non-ionic synthetic polymer of sucrose, molecular weight 400 kilodaltons.

Analytical reagents used were reagent grade or better. Research grade dimethylformamide was used for moisture content analysis.

Sample Preparation and Handling:

Spray-dried powders were stored and prepared for analytical testing in a glove box maintained with dry air atmosphere. During powder transfer, the dry box was maintained at less than 5% relative humidity.

Physical Methods:

Moisture content

The water content of the powder formulations was determined by coulometric Karl Fischer titration using a Mitsubishi Model CA-06 moisture meter. A 5–10 mg aliquot of powder was dissolved in 1 ml of dimethylformamide (DMF). The sample was then injected into the titration cell of the moisture meter.

Powder particle size distribution

The particle size distribution (PSD) of the spray dried powder samples was measured with a Horiba CAPA-700 centrifugal sedimentation particle size analyzer. Approximately 2–3 mg of powder was suspended in 2–3 ml of Sedisperse A-11 (Micromeritics, Norcross, Ga.) and sonicated briefly (5 minutes) before analysis. The instrument was configured to measure a particle size range of 0.4 to 10 μm in diameter (MMD). A particle density of 1.44 g/cm^3 was used for analyzing these powders.

Aerosol Methods:

Delivered dose assay

Delivered dose assay was performed to determine the efficiency and reproducibility of pulmonary delivery of the dispersible dry powder antibody composition. The device used was similar to devices disclosed in WO96/09085. The delivered dose was measured by collecting the aerosol on a filter placed over the mouthpiece of the chamber of the device. The powder sample was collected on a glass fiber filter (Gelman, 47 mm diameter) by drawing the aerosol from the chamber at an airflow of 30 L/minute for 2.5 seconds. Delivered dose efficiency was calculated by dividing the mass of the powder collected on the filter by the mass of the powder in the blister pack. Each result was the average of five replicate measurements.

Aerosol particle size distribution

The aerosol particle size distribution was obtained using an eight stage cascade impactor (Graseby Andersen, Smyrna, Ga.). The impactor air-flow was set to 28.3 L/min., the calibrated flow-rate for the instrument. For each run, 5 blister packs filled with approximately 5 mg of powder was dispersed from the inhaler. The particle size was determined by weighing the powder on the impactor plates and evaluating the results on a log-probability plot. Both the mass median aerodynamic diameter (MMAD) and the mass fraction less than 5 μm were determined from the log-probability plot.

The mass fraction of the aerosol powder less than 3.3 μm was obtained using a 2-stage (3.3 μm and 0.4 μm cut size) cascade impactor (short-stack Andersen). The impactor airflow was set to 28.3 L/minute, the calibrated operating flow rate of the instrument. For each measurement, one blister pack filled with a known weight of powder was dispersed from the inhaler. The resultant aerosol was drawn from the device chamber into the cascade impactor. The particle size fractions were determined by weighing the powder on the impactor filters. The % of mass <3.3 μm was determined based upon the weights of those filters.

Biophysical methods:

Attenuated Total Reflectance Fourier Transform—IR (ATR FT-IR)

Infrared (IR) spectroscopy can provide information regarding the secondary structure of proteins. The majority of the protein structural information, however, has been obtained from one absorbance originating primarily from the amide C=O stretching vibration: the amide I band. The sensitivity to variations in both geometric arrangements of atoms and hydrogen bonding enables infrared spectroscopy to discriminate between the various secondary structures incorporated within the three-dimensional organization of peptides and proteins, e.g., helical, extended sheet, disordered and turns. Solid samples, e.g., powders, which are not transparent are analyzed using ATR-IR. The basis for ATR-IR is that the IR beam enters an optically transparent medium, in this case Germanium crystal, on which the sample is layered. Comparison FT-IR spectra of spray dried and lyophilized powders were analyzed for differences which would indicate conformational changes due to spray drying. FIG. **1** shows the FT-IR spectra of lyophilized IgG and spray dried IgG. No significant difference in the spectra shape or peak maxima were noted, indicating that the spray dried IgG powder retained its conformational integrity. FIG. **2** shows that both a 100% IgG powder formulation and an

IgG:sucrose:citrate (70:20:10) powder retained native conformation of antibody.

Intrinsic Tryptophan Fluorescence

For most proteins, aromatic amino acid residues like tryptophan, tyrosine and phenylalanine contribute to the intrinsic fluorescence when excited at 280 nm. The intrinsic fluorescence in proteins is strongly dependent on the local environment of these fluorophores. A shift in the fluorescence maximum to a longer wavelength is observed when the environment changes from non-polar to polar. Antibodies, which have tryptophan residues in a relatively non-polar environment in the native state, have a fluorescence maximum at 337 nm which is shifted to 350 nm upon unfolding of the protein. The intrinsic fluorescence of reconstituted antibody powder formulations were measured using a SPEX-fluoromax fluorescence spectrophotometer. The samples were excited at 280 nm and fluorescence maxima measured. FIG. **3** shows fluorescence scans of reconstituted IgG:mannitol:citrate (70:20:10), IgG:PVP:citrate (70:20:10) and 100% IgG powders and a solution of IgG which had been unfolded by the denaturant guanidinium HCL. The scans of the reconstituted powder formulations show no shift in wavelength, indicating no unfolding of antibody. In contrast, the scan of the denatured IgG solution shows a wavelength shift indicative of unfolding.

UV turbidity assay

The UV turbidity assay was carried out to monitor physical stability of the protein and also to detennine the yield upon reconstitution of the dry-powder in water. Suitable formulations must reconstitute in water without turbidity. The absorbance at 400 nm is a measure of the extent of aggregation (insoluble aggregates) in the solution. FIG. **4** shows that no aggregation was seen with an IgG:mannitol::citrate (70:20:10) formulation.

Dynamic Light Scattering (DLS)

Dynamic Light Scattering (DLS) measures light scattered from particles based on Brownian motion, the interaction of particles with solvent molecules. The instrument (Malvern Instruments) detects the fluctuation of light intensity using a digital correlator. The correlation functions are fitted into an analytical program to calculate the particle size distribution. DLS was used to measure soluble aggregates in reconstituted antibody powder formulations. Samples of individual blister packs were combined to give approximately 2–4 mg of powder per ml. These samples were reconstituted in water and centrifuged at 10,000 rpm for 5 minutes to remove any dust particles, which interfere with readings. The samples were decanted and placed in a cylindrical quartz cell for analysis. A semi-quantitative method of particle size determination by light intensity was developed, using a multimodal method of data analysis supplied by Malvern Instruments. Measurements were taken at a range of angles from 75° to 120°. Larger particles are more easily detectable at smaller angles, while smaller particles are more readily detected at larger angles. The IgG monomer has been reported to have a diameter of 11 nm, as measured by DLS (Singh et al., Biopolymers 31: 1387–1396 (1991)).

SEC-HPLC

SEC-HPLC was used to measure soluble aggregates in reconstituted antibody powder formulations. An HPLC system equipped with a TSK-3000 column was used. The mobile phase consisted of 0.05M KH_2PO_4 buffer (pH 6.8), 0.1 M KCl and 0.0015 M NaN_3. The assay results were obtained by determining the relative area for each peak in the chromatogram for spray dried material relative to lyophilized material. Preferred formulations showed less than 5% aggregates by SEC-HPLC.

SDS-PAGE

Silver stain SDS-PAGE analysis was used to evaluate reconstituted powder formulations for covalent aggregates of the antibodies and to confirm SEC-HPLC methods. SDS-PAGE analysis was carried out using a Pharmacia Phast system consisting of discontinuous 8 to 25% gradient Phast gels. Spray dried antibody powder formulations were reconstituted with purified water, then further diluted with SDS-PAGE buffer to an antibody concentration of 1 mg/ml. Samples were incubated in 10% SDS non-reducing sample buffer at 37° C. for 30 minutes, then 4 μg samples were loaded into each well. The gels were run and silver stained using Pharmacia standard procedures for 8 to 25% SDS-PAGE gels.

Formulation preparation:

A 100% formulation of antibody was prepared by dissolving 5 mg of IgG in 1.0 ml of deionized water. The pH of the protein solution was measured to be about 6.5.

A 90% formulation of antibody was prepared by dissolving 4.5 mg of IgG in 1.0 ml of 2mM citrate buffer. The pH of the protein solution was measured, and was generally found to be about 6.5.

A 70% formulation of antibody was prepared by dissolving 3.5 mg of antibody and 1 mg/ml of excipient in 1.0 ml of 2 mM citrate buffer. The pH of the protein solution was measured to be about 6.5.

The various classes of excipients used were as follows:

Sugar excipients: sucrose, lactose, mannitol, raffinose and trehalose.

Polymeric excipients: Ficoll and PVP.

Protein excipients: HSA.

Powder processing:

Dry powders of the above formulations were produced by spray drying using a Buchi Spray Dryer using the following parameters:

Temperature of the solution 4–6° C.

Inlet temperature 98–105° C.

Feed rate 5.0 ml/min.

Outlet temperature 64–67° C.

Atomizer pressure 40 psi

Cyclone coolant temperature 30° C.

Once the aqueous mixture was consumed, the outlet temperature was maintained at 67° C. for 10 minutes by slowly decreasing the inlet temperature to provide secondary drying.

Process suitability was assessed by evaluating the antibody powders for moisture content, particle size distribution, delivered dose, aerosol efficiency and antibody integrity.

Before preparing the antibody powders, solution formulations were analyzed to demonstrate that the protein is stable in solution over the process period during spray-drying. The antibody solutions were atomized to investigate protein aggregation in the high shear of the pneumatic atomizer. The protein stability was assayed by UV and SEC-HPLC. The absence of insoluble protein aggregates as determined by UV in the 350–400 nm region of the spectrum indicated that antibody did not aggregate or precipitate during atomization.

EXAMPLE 1

100% IgG Formulation For Pulmonary Delivery

Bulk lyophilized IgG was formulated and spray dried as described above. The 100% IgG dry powder was analyzed

for moisture as described above and found to contain 2.5–3.5% moisture.

The particle size distribution was measured by centrifugal sedimentation as described above and was determined to be 1.3 μm to 1.5 μm MMD. The delivered dose of the IgG powder composition was measured as described above and was determined to be 60 to 62% of the total powder (5.0 mg) loaded into the device. The aerosol particle size distribution, measured as described above was determined to be 4.1 μm MMAD, with 61.6% of the particles <5.0 μm in diameter.

The antibody integrity was assayed by SEC-HPLC and dynamic light scattering (DLS) as described above. The powder did not reconstitute easily in water (47% reconstitution), so no UV measurement was possible. The mean diameter of particles was found to be 18 nm by DLS. The size distribution indicated the presence of a small population of soluble aggregates. We found 7.5% soluble aggregates by SEC-HPLC in the reconstituted spray dried sample. ATR FT-IR and intrinsic fluorescence assays, performed as described above, indicated no conformational change in antibody from spray drying. SDS-PAGE results showed the presence of covalent aggregates.

EXAMPLE 2

90% IgG Formulation For Pulmonary Delivery

Bulk lyophilized IgG was formulated and spray dried as described above. The 90% IgG dry powder composition contained 90% IgG and 10% citrate. The formulation contained 2–2.5% moisture.

The particle size distribution was determined by centrifugal sedimentation to be 1.3 μm to 2.0 μm MMD, with 98% of the particles less than 5 μm. The delivered dose of the IgG powder was determined to be 46% of the total powder (5.0 mg) loaded into the device. The aerosol particle size distribution was determined to be 1.5 μm MMAD, with 99% of the particles <5.0 μm in diameter.

The powder gave about 66% reconstitution in water. The antibody integrity was assayed by SEC-HPLC. About 15% of soluble aggregates was detected in the reconstituted sample after spray-drying. The fluorescence maximum for the reconstituted sample was around 337 nm, indicating no alteration in antibody conformation after spray drying.

EXAMPLE 3

70% IgG Formulation Containing Carbohydrate Excipient

Bulk lyophilized IgG was formulated and spray dried as described above. A 70% formulation was achieved by dissolving 3.5 mg of IgG and 1 mg sucrose in 1.0 ml of 2 mM citrate buffer. The 70% IgG dry powder composition contained 70% IgG, 20% sucrose and 10% citrate. The pH of the resulting solution was determined to be about 6.5. The formulation contained 2–2.5% moisture.

The particle size distribution was determined by centrifugal sedimentation to be 1.0 μm to 1.5 μm MMD, with 97% of the particles less than 5 μm. The delivered dose of the IgG powder was determined to be 45 to 52% of the total powder (5.0 mg) loaded into the device. The aerosol particle size distribution was determined to be 3.2 μm MMAD, with 67% of the particles <5.0 μm in diameter and 45% under 3.3 μm.

The antibody integrity was assayed by UV and SEC-HPLC. The powder reconstituted well, with complete recovery of antibody and absence of aggregates as shown by UV. About 3–4% of soluble aggregates were detected by SEC-HPLC in the reconstituted sample after spray-drying. The fluorescence maximum for the reconstituted sample was around 337 nm, indicating no alteration in antibody conformation after spray drying.

EXAMPLE 4

70% IgG Formation Containing Polymeric Excipient

Bulk lyophilized IgG was formulated and spray dried as described above. A 70% formulation was achieved by dissolving 3.5 mg of IgG and 1 mg of PVP in 1.0 ml of 2 mM citrate buffer. The pH of the protein solution was measured to be at 6.7. The 70% IgG dry powder composition contained 70% IgG, 20% PVP and 10% citrate. The formulation contained 1.5–2% moisture.

The particle size distribution was determined by centrifugal sedimentation to be 1.7 μm to 2.0 μm MMD, with 97% of the particles less than 5 μm. The delivered dose of the IgG powder was determined to be 62% of the total powder (5.0 mg) loaded into the device. The aerosol particle size distribution showed that 41% of the particles were <3.3 μm in diameter.

Antibody integrity was assayed by UV. The absence of insoluble protein aggregates as determined by UV in the 350–400 nm region of the spectrum indicated that IgG did not aggregate or precipitate during spray-drying. The fluorescence maximum for the reconstituted sample was around 337 nm, indicating no alteration in antibody conformation after spray drying.

EXAMPLE 5

70% IgG Formulation With Protein Excipient

Bulk lyophilized IgG was formulated and spray dried as described above. A 70% formulation was achieved by dissolving 3.5 mg of IgG and 1 mg HSA (Miles-Pentex) in 1.0 ml of 2 mM citrate buffer. The pH of the protein solution was measured to be at 6.2. The 70% IgG dry powder composition contained 70% IgG, 20% HSA and 10% citrate. The formulation contained 2.8–3.3% moisture.

The particle size distribution was determined by centrifugal sedimentation to be 1.3–1.5 μm MMD, with 100% of the particles less than 5 μm. The delivered dose of the IgG powder was determined to be 70 to 75% of the total powder (5.0 mg) loaded into the device. The aerosol particle size distribution was determined to be 3.8 μm MMAD, with 68% of the particles <5.0 μm in diameter.

Antibody integrity was assayed by UV and SEC-HPLC. The powder did not reconstitute easily, with only about 35% recovered after reconstitution based on UV analysis. About 9% of soluble aggregates was determined by SEC-HPLC. No fluorescence determination was performed, due to interference that would result from the presence of another protein, i.e., HSA.

EXAMPLE 6

90% IgG Formulation With Surfactant Excipient

Bulk lyophilized IgG was formulated and spray dried as described above. A 90% formulation was achieved by dissolving 4.5 mg of IgG in 1.0 ml of 2 mM citrate buffer containing 0.05% BAC. The pH of the protein solution was measured to be at 6.5. The above 90% IgG dry powder

composition contained 89.95%lo IgG, 0.05% BAC and 10% citrate. The formulation contained 3–3.5% moisture.

The particle size distribution was determined by centrifugal sedimentation to be 1.4 μm MMD, with 100% of the particles less than 5 μm. The delivered dose of the IgG powder was determined to be 45% of the total powder (5.0 mg) loaded into the device. The aerosol particle size distribution was determined to be 3.6 μm MMAD, with 69% of the particles <5.0 μm in diameter.

The IgG-surfactant powder gave a turbid-looking solution upon reconstitution.

EXAMPLE 7

100% IgA Formulation

IgA obtained from Sigma was preformulated in 10 mM Tris and 100 mM NaCl, pH 7.4. It was dialyzed against de-ionized water overnight at 4° C. The dialyzed protein was concentrated using a filtron unit to 5 mg/ml. The pH of the resulting solution was determined to be 6.9. The solution was spray dried as described above. The 100% IgA dry powder contained 3–3.5% moisture.

The particle size distribution was determined by centrifugal sedimentation to be 1.22 μm MMD, with 97% of the particles less than 5 μm. The delivered dose of the IgA powder was determined to be 50–52% of the total powder (5.0 mg) loaded into the device.

Antibody integrity was assayed by UV and dynamic light scattering. The absence of insoluble protein aggregates as determined by UV in the 350–400 nm region of the spectrum indicated that IgA did not aggregate or precipitate during atomization.

EXAMPLE 8

70% IgA Formulation Containing Protein Excipient

IgA obtained from Sigma was preformulated in 10 mM Tris and 100 mM NaCl, pH 8.0. It was dialyzed against 2 mM citrate buffer, pH 6.5 overnight at 4° C. 40 mg of HSA (Miles Pentex) was dissolved in 30 ml of dialyzed IgA solution (4.16 mg/ml) and the resulting solution spray dried as described above. The 70% IgA dry powder composition contained 70% IgA, 20% HSA and 10% citrate. The formulation contained 2.6% moisture.

The particle size distribution was determined by centrifugal sedimentation to be 1.22 μm MMD, with 97% of the particles less than 5 μm. The delivered dose of the IgA powder was determined to be 65% of the total powder (5.0 mg) loaded into the device.

EXAMPLE 9

70% IgG Formulation Containing Carbohydrate Excipient

Bulk lyophilized IgG was formulated and spray dried as described above. A 70% formulation was achieved by dissolving 3.5 mg of IgG and 1 mg mannitol in 1.0 ml of 2 mM citrate buffer. The 70% IgG dry powder composition contained 70% IgG, 20% mannitol and 10% citrate. The pH of the resulting solution was determined to be about 6.5. The formulation contained 1.7% moisture.

The particle size was determined by centrifugal sedimentation to be 1.9 μm MMD, with 100% of the particles less than 5 μm. The delivered dose of the IgG powder was determined to be 53%±4% of the total powder (5.0 mg) loaded into the device. The aerosol particle size distribution showed that 45% of the particles were <3.3 μm in diameter.

The antibody integrity was assayed by UV and SEC-HPLC. The powder reconstituted well, with complete recovery of antibody and absence of aggregates as shown by UV. About 3–4% of soluble aggregates were detected by SEC-HPLC in the reconstituted sample after spray-drying. The fluorescence maximum for the reconstituted sample was around 337 nm, indicating no alteration in antibody conformation after spray drying. SDS-PAGE results showed the presence of covalent aggregates.

EXAMPLE 10

70% IgG Formulation Containing Polymeric Excipient

Bulk lyophilized IgG was formulated and spray dried as described above. A 70% formulation was achieved by dissolving 3.5 mg of IgG and 1 mg of Ficoll-4000 in 1.0 ml of 2 mM citrate buffer. The pH of the protein solution was measured to be at 6.7. The 70% IgG dry powder composition contained 70% IgG, 20% Ficoll and 10% citrate. The formulation contained 1.9% moisture.

The particle size distribution was determined by centrifugal sedimentation to be 1.78 μm MMD, with 100% of the particles less than 5 μm. The delivered dose of the IgG powder was determined to be 62% of the total powder (5.0 mg) loaded into the device.

EXAMPLE 11

Effect Of Excipients On DDE Of Spray Dried IgG Powders

The delivered dose efficiency (DDE) of the spray dried IgG powders varied considerably, depending on the type of excipient used. The 100% IgG powders showed a delivered dose efficiency of about 60%. The use of sugars as excipients decreased the delivered dose efficiency to about 40–55%. Addition of polymeric excipients such as Ficoll and PVP did not alter the delivered dose efficiency (with respect to the 100% IgG). Results are presented in Table 1.

HSA is a commonly used protein excipient for parenteral delivery. An increase in the delivery efficiency of powders of small molecules and proteins upon addition of HSA has been observed. Addition of HSA to the solutions used to form spray dried IgG powders resulted in an increase in the DDE, although the increase seemed to be independent of the HSA concentration (5–20%). The mass median aerodynamic diameters (MAD) ranged from 2.5–4 μm for all powders tested.

TABLE 1

Effect of Excipients on DDE of Spray Dried IgG Powders

Powder Formulation	Delivered Dose Efficiency	Relative Standard Deviation
100% IgG	60	8
100% IgG	62	1
90% IgG, 10% Citrate	37	7
90% IgG, 10% Citrate	44	9
70% IgG, 20% Sucrose, 10% Citrate	53	6
40% IgG, 20% Sucrose, 10% Citrate	52	5
70% IgG, 20% Mannitol, 10% Citrate	53	4
70% IgG, 20% Trehalose, 10% Citrate	49	7

TABLE 1-continued

Effect of Excipients on DDE of Spray Dried IgG Powders

Powder Formulation	Delivered Dose Efficiency	Relative Standard Deviation
70% IgG, 20% PVP, 10% Citrate	63	3
70% IgG, 30% Citrate	48	18
70% IgG, 20% Lactose, 10% Citrate	48	5
70% IgG, 20% Raffinose, 10% Citrate	42	4
70% IgG, 20% Ficoll 400, 10% Citrate	62	6
70% IgG, 20% HSA, 10% Citrate	70	5
70% IgG, 30% HSA	75	2
70% IgG, 20% HSA, 10% Citrate	71	6
70% IgG, 20% HSA, 10% Citrate	73	10
85% IgG, 15% HSA	79	5
95% IgG, 5% HSA	80	1
85% IgG, 15% HSA	77	4
85% IgG, 15% HSA	79	2
85% IgG, 15% HSA	71	7
95% IgG, 5% HSA	76	5

EXAMPLE 12

Rugosity Of Antibody Powders

The rugosity of certain dry powder antibody compositions of the present invention is presented in Table 2. Results showed that all antibody powders and a powder composed of 100% HSA had rugosity of at least 2.

TABLE 2

Rugosity of Antibody Powders

Powder Formulation	Rugosity	DDE
100% IgG	2.02	60
IgG + Citrate	2.40	51
100% HSA	3.37	81.4
IgG + HSA	2.93	75.3

Modification of the above-described modes of carrying out the various embodiments of this invention will be apparent to those skilled in the art following the teachings of this invention as set forth herein. The examples described above are not limiting, but are merely exemplary of this invention, the scope of which is defined by the following claims.

The disclosure of each publication, patent or patent application mentioned in this specification is hereby incorporated by reference to the same extent as if each individual publication, patent or patent application were specifically and individually indicated to be incorporated by reference.

We claim:

1. An antibody-based dry powder composition for pulmonary delivery comprising a predetermined amount of antibody which is non-aggregated and is in its native conformation in respirable dry powder particles having a particle size less than 10 micrometers, wherein said composition has a delivered dose efficiency of at least about 30%.

2. The composition of claim **1** which is dispersible.

3. The composition of claim 1 wherein the antibody is selected from the group consisting of IgG, IgA and IgM antibodies.

4. The composition of claim **1** which further comprises a pharmaceutically acceptable excipient or carrier.

5. The composition of claim **4** wherein the carrier or excipient is selected from the group consisting of sugars, alditols, organic salts, amino acids, polymers, proteins and peptides.

6. The composition of claim **1** wherein the antibody comprises at least about 40% by weight of the composition.

7. The composition of claim **1** wherein the particles in the powder have a particle size range of from about 0.4 μm to about 5 μm MMD.

8. The composition of claim **1** wherein the particles have an MMAD of less than about 5 μm.

9. The composition of claim **1** which contains less than about 10% by weight moisture.

10. A method for preparing the composition of claim **1** comprising:

a) mixing the antibody with a solvent to form a solution or suspension; and

b) spray drying the mixture formed in step a) under conditions which provide a respirable dry powder, wherein the antibody is non-aggregated and is in its native conformation.

11. The method of claim **10** further comprising the step of adding a pharmaceutically acceptable excipient or carrier prior to spray drying.

12. The method of claim **10** wherein the solvent is selected from the group consisting of water and ethanol.

13. A method of treating or preventing a condition in an animal which condition may be prevented or alleviated by an antibody, the method comprising pulmonary administration of a therapeutically effective amount of the composition of claim **1** to an animal susceptible to or suffering from the condition.

14. The method of claim **13** wherein the condition is selected from the group consisting of inflammation, allergy, cancer, bacterial infection and viral infection.

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